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MYCOLOGIA

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PARASITIC AND SAPROPHYTIC PHYCOMYCETES WHICH INVADE PLANKTONIC ORGANISMS. II. A NEW SPECIES OF DANGEARDIA WITH NOTES ON OTHER LACUSTRINE FUNGI

R. A. PATERSON [1]

(WITH 27 FIGURES)

From 1953 to 1956 an investigation of planktonic Phycomycetes was conducted in certain Michigan waters. Several chytridiaceous parasites which infect phytoplankters were found and subsequently reported (Paterson, **1956, 1957**). It is the purpose of this communication to give an account of parasitic chytrids of planktonic algae not considered in the two previous papers and to report on some of the other aquatic fungi encountered during the course of this work. One new species is described and several incompletely known chytridiaceous parasites of planktonic algae are discussed. In addition, one chytrid which infects a zooplankter, two saprolegniaceous fungi found on phytoplankters, and two species of this same group found on zooplankters are presented.

Dangeardia ovata sp. nov. (FIGS. 1–6)

Sporangium ovoideum, in massa gelatinosa hospitis immersum, 9–14.5 μ longum, 6.5–10.5 μ crassum; membrana laevi, hyalina; rhizoideis ramosis, plerumque ex

[1] This paper forms a part of a thesis accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University of Michigan.

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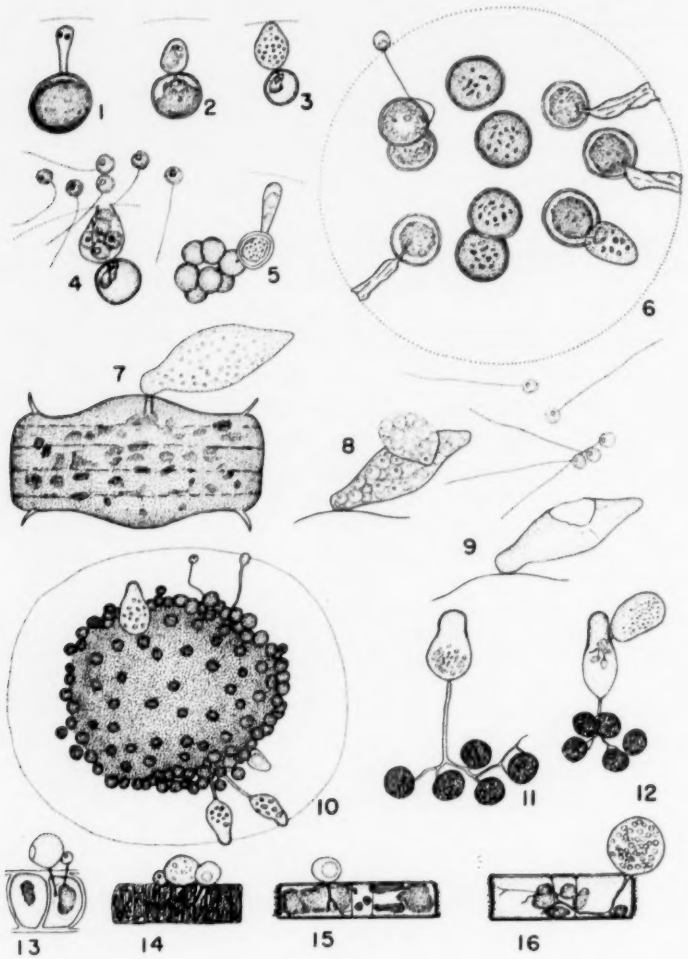


FIGS. 1–16.

FIGS. 1–6. *Dangeardia ovata* n. sp. on *Sphaerocystis schroeteri* Chod. 1, 2, 3. Stages in development of sporangia. 4. Discharging sporangium with zoospores. 5. Resting spore in host sheath resting on surface of a host cell. 6. Developing thalli and discharged sporangia in host, × 860. FIGS. 7–9. Parasite on *Stephanodiscus* sp. 7. Nearly mature thallus on host. 8. Discharging sporangium with zoospores in vesicle. 9. Empty sporangium with discharged zoospores nearby, × 860. FIGS. 10–12. Parasite of *Microcystis* sp. 10. Thalli in various stages of development

trunco orientibus. Zoosporae sphaericae, 3–3.5 μ diametro, singulatim emergentes cito saltantes. Sporae durantes globosae, in massa gelatinosa hospitis immersae, tubo germinationis longo, persistenti praeditae, membrana 6.5–9 μ crassa, laeve, intus granulosa. Germinatio nondum observata.

Sporangium ovoid, embedded in the gelatinous matrix of the host, 9–14.5 μ long by 6.5–10.5 μ in diameter, wall smooth, colorless; rhizoids branched, usually arising from a main axis; zoospores spherical, 3–3.5 μ in diameter, emerging singly and swimming away at once; resting spore globose, in the gelatinous host sheath, with a long persistent germ tube and a thick smooth wall, contents finely granular, 6.5–9 μ in diameter, germination not observed.

Parasitic in *Sphaerocystis schroeteri* Chod. in plankton from Whitmore Lake, Washtenaw County, Michigan, May, 1955, and Frains Lake, Washtenaw County, Michigan, July, 1955. The holotype is a collection of several individuals of *Dangeardia ovata* which is preserved with a mixture of other planktonic organisms on a microscopic slide. The slide was prepared from a sample of plankton taken from Whitmore Lake, Washtenaw County, Michigan, on May 5, 1955, and has been deposited at the Mycological Herbarium of the Plant Industry Station in Beltsville, Maryland.

The sequence of events in the development of this chytrid is that characteristic for species of *Dangeardia*. The encysted zoospore, at rest on the surface of the gelatinous envelope of the host, sends a delicate germ tube into the thick mucilage sheath (FIG. 6). Growth of the tube continues until entrance is gained into the host-cell contents, where a branched rhizoidal system is produced. Shortly after rhizoid formation the contents of the algal cell recede from the wall and turn brown. Meanwhile, the tenuous penetration tube in the gelatinous matrix of the host gradually broadens until it is almost cylindrical (FIG. 1). It continues to enlarge and subsequently forms an ovoid sporangium 6.5 to 10.5 μ wide by 9 to 14.5 μ long (FIGS. 2, 3). Its protoplasm is at first granular, interspersed with refractive droplets which eventually coalesce to form the oil globules of the zoospores. The swarmers at maturity are spherical, 3 to 3.5 μ in diameter, and posteriorly uniflagellate. They are discharged singly from the sporangium after the deliquescence of its apex which is formed from the original cyst of the zoospore (FIG. 4).

in a host colony, × 355. 11. Developing sporangia on host. 12. Epibiotic hyperparasite on chytrid parasitizing *Microcystis* sp., × 860. FIG. 13. Empty sporangium at the left, showing lateral discharge pore; developing thallus at the right, of a chytridiaceous parasite of *Ulothrix* sp., × 860. FIG. 14. Developing thalli of a chytridiaceous parasite on *Oscillatoria* sp., × 860. FIGS. 15, 16. Parasite of *Melosira* sp. 15. Developing thallus. 16. Nearly mature sporangium, × 860.

During discharge the delicate wall of the sporangium begins to shrink and after all the zoospores have emerged, collapses (FIG. 6).

The resting spore is epibiotic on an algal cell and, like the sporangium, is embedded in the gelatinous envelope of the host (FIG. 5). It has a thick smooth wall and remains attached to the persistent clavate germ tube. The resting-spore wall is consistently smooth as in *D. laevis* Sparrow and Barr (**1955**) and is never found to possess the spines or granules that are characteristic of *D. mammillata* Schröder (**1898**). No pairing of the zoospores such as was described in *D. mammillata* by Canter (**1946**) preceding resting-spore formation was observed in the present species.

The ovoid shape of the sporangium of *D. ovata* is the most striking feature distinguishing it from *D. mammillata* and *D. laevis*. In both of the latter species the sporangium is distinctly flask-shaped. A further structural difference is that in *D. ovata* the rhizoids are branched, whereas they are short, unbranched, bushy or tufted in *D. mammillata* and short and unbranched in *D. laevis*. Finally, there is a difference in hosts. *D. ovata*, so far as is known, infects only *Sphaerocystis schroeteri*, whereas the hosts for *D. mammillata* and *D. laevis* are *Pandorina* and *Eudorina*, and *Gloeodinium*, respectively.

The fungus has been placed in the genus *Dangeardia* because of its inoperculate sporangium, the method of formation of the sporangium by the swelling of the germ tube, and its epibiotic resting spore. The original generic description by Schröder states that the resting spore is endobiotic. However, subsequent studies by Canter (**1946**) have shown it to be, in fact, epibiotic.

RHIZOPHYDIUM MEGARRHIZUM Sparrow (FIGS. 21, 22)

Sparrow (**1943**) segregated a parasite of *Oscillatoria* from *Rhizophydium subangulosum* (Braun) Rabenhorst (**1868**) as *R. megarrhizum*, on the basis of differences in the rhizoidal system. The latter has a broad tubular rhizoidal system whereas the former has delicate rhizoids. Organisms from two southern Michigan lakes were identified as belonging to Sparrow's species. One from Whitmore Lake, Livingston County, was found on *Lyngbya* sp. during September, 1954, and the other from Portage Lake, Washtenaw County, parasitized *Aphanizomenon flos-aquae* (L.) Ralfs in January, 1955.

Although zoospore discharge was not observed in the organism parasitizing *Lyngbya* sp., the species is so distinctive that it is impossible not to recognize it. The sporangium was ellipsoidal, 14.5 to 32 μ long by 7 to 8.5 μ wide (FIG. 21). The rhizoids were broad, undulating,

usually forked into two branches, extending through many cells of the host trichome, and up to 50 μ in length. The sporangia of the parasite of *A. flos-aquae* are 6.5 to 9 μ in diameter and spherical at first, becoming somewhat angular at maturity as in *Rhizophydium subangulosum* (FIG. 22). The rhizoids, which were observed only after clearing the host cell with lacto-phenol, extended through several cells of the host and were coarse, undulate, and occasionally branched. They were not as delicate as in the related *R. subangulosum* or quite as "mycelial" as in *R. megarrhizum*. They were, however, coarse and may be considered as intermediate in this respect between the two, and bear closest resemblance to those found in *R. megarrhizum*.

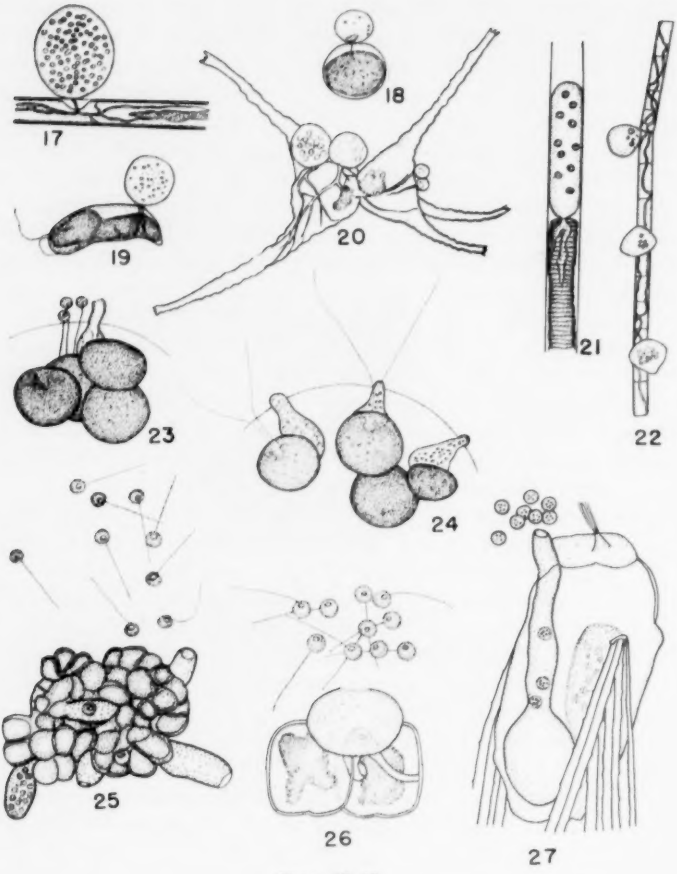
Seven chytridiaceaous planktonic parasites were found in which observations were too incomplete to indicate their precise taxonomic relationships. This was due to their rarity and, although water samples were concentrated many times, they often contained only a few such parasites. Nevertheless, such characteristics as their developmental sequence and nature of their rhizoidal system support the view that they are all species of either *Rhizophydium* or *Chytridium*.

SPECIES 1. (FIG. 18) This chytrid was found infecting actively motile *Chlamydomonas* cells in Frains Lake, Washtenaw County, Michigan, during February, 1954.

The spherical sporangium, up to 9 μ in diameter, is formed by the enlargement of the encysted zoospore on the cell wall of the host. The rhizoidal system consists of an unbranched tube which arises from the base of the epibiotic sessile sporangium and penetrates the host cell contents, causing them to shrivel and turn brown. The present organism is possibly referable to *Rhizophydium transversum* (Braun) Rabenhorst (**1868**), which is also parasitic on species of *Chlamydomonas*. Although the sporangia in *R. transversum* are broadly ellipsoidal or fusiform, each with a pronounced apical papilla, they are at first spherical. The possibility exists, therefore, that the spherical sporangia observed in the Frains Lake parasite were immature ones of Braun's species. Evidence that only the young zoosporangia were observed in the present fungus lies in the fact that no stage was observed in which the sporangial contents contained numerous oil globules of uniform size and spacing, a condition typical of mature chytridiaceous sporangia.

SPECIES 2. (FIG. 19) This fungus was found parasitic on *Cryptomonas* sp. from Frains Lake, Washtenaw County, in January, 1956.

The sessile sporangium is spherical, up to 12 μ in diameter, with a coarse, branched, tapering rhizoidal system. Other than the fact that the present fungus is parasitic on *Cryptomonas* sp., there is no out-



FIGS. 17–27.

FIG. 17. Sporangium of a chytrid parasitizing *Closterium* sp., × 688. FIG. 18. Developing thallus of a chytrid parasitic on *Chlamydomonas* sp., × 688. FIG. 19. Sporangium of a chytrid parasitizing *Cryptomonas* sp., × 688. FIG. 20. Developing thalli of a chytrid parasitic on *Staurastrum* sp., × 688. FIGS. 21, 22. *Rhizophydium megarrhizum* Sparrow. 21. Thallus on *Lyngbya* sp. 22. Sporangia on *Aphanizomenon flos-aquae* (L.) Ralfs; host cleared with acetocarmine, × 688. FIGS. 23, 24. *Dangeardia* sp. in a colony of *Eudorina elegans* Ehr. 23. Germinated zoospores and discharged sporangium. 24. Sporangia in varying stages of zoospore maturity, × 688. FIG. 25. Parasite of *Botryococcus braunii* Kütz.; developing and discharging sporangia with zoospores, × 284. FIG. 26. *Dangeardia* sp. on

standing distinguishing morphological characteristic which would refer it to any particular species. The only other parasite belonging to the genus *Rhizophydium* which parasitizes a member of the genus *Cryptomonas,* so far as is known, is *Rhizophydium simplex* (Dangeard) Fischer (**1892**). The sporangium of *R. simplex,* however, is narrowly pyriform whereas in the present species it is spherical. No species belonging to *Chytridium* have as yet been described as parasitic on *Cryptomonas.*

SPECIES 3. (FIG. 20) During October, 1954, and July, 1955, a parasite was found on a member of the Desmidiaceae, *Staurastrum* sp., in Frains Lake, Washtenaw County, Michigan.

In the developmental sequence of this chytrid, the zoospore cyst on the host cell sends out a germ tube which enters the alga and forms a branched rhizoidal system. Its elements, which arise from a more or less developed main axis and taper terminally, penetrate the chromatophores and cause them to shrink and turn yellow-brown. Meanwhile, the encysted zoospore expands into a spherical to slightly subspherical zoosporangium 9 to 10.5 μ in diameter, containing at first numerous refractive granules which later coalesce to form several uniform oil globules.

Inasmuch as zoospore discharge was not observed and there are no striking or unusual morphological features, positive identification of the present species is not possible. Nevertheless, it most closely resembles *Rhizophydium globosum* (Braun) Rabenhorst (**1868**) in its spherical sporangium, branched rhizoidal system, and its presence on the host *Staurastrum* sp. One difference between the two is concerned with the sporangial wall, which in Braun's species is "double contoured" whereas in the present organism it is single and delicate. The *Staurastrum* parasite also resembles *R. difficile* Canter (**1954**) in its spherical thin-walled sporangium and in its host. They differ, however, in the nature of their rhizoidal systems. *R. difficile* has much-branched rhizoids of limited extent whereas, in the present fungus, the branched rhizoidal system ramifies throughout most of the cell.

SPECIES 4. (FIG. 17) Neither the developmental stages nor zoospore discharge was observed in this parasite of *Closterium* sp., which was found in April, 1955, in Crooked Lake, Washtenaw County, Michigan. The zoosporangium was sessile, spherical to broadly ovoid, up to 21 μ in diameter, and was presumably formed by an enlargement of the

Hyalotheca sp.; empty sporangium with discharged spores nearby, host sheath not visible, × 688. FIG. 27. Parasite of *Polyarthra* sp.; some with zoospores still within the sporangium or at the surface of its discharge tube, × 284.

zoospore cyst. Arising from the base of the sporangium was an endobiotic branched rhizoidal system.

Other parasites found on members of the genus *Closterium* which resemble the present species are *Rhizophydium globosum* (Braun) Rabenhorst (**1868**) and *Chytridium schenkii* (Schenk) Scherffel (**1926**). It resembles *R. globosum* in the size and shape of the sporangium, which is spherical, and in its branched rhizoidal system. The two species differ, however, in the fact that the sporangial wall of *R. globosum* is double-contoured, whereas in the present organism it is single-contoured and delicate. The Crooked Lake parasite resembles *C. schenkii* in the ovoid shape of the sporangium and in the branched rhizoidal system. The sporangium of *C. schenkii*, however, has a prominent apical papilla and a persistent unenlarged functioning zoospore cyst, which is not present in the organism under consideration.

SPECIES 5. (FIG. 14) A parasite of *Oscillatoria* sp. which might be considered related to *Rhizophydium subangulosum* (Braun) Rabenhorst (**1860**) or *R. oscillatoriae* Jaag and Nipkow (**1951**) was found in Frains Lake, Washtenaw County, Michigan, in June, 1955.

This organism resembles *R. subangulosum* and *R. oscillatoriae* in its delicate rhizoidal system, host, and its spherical immature sporangium. At maturity the sporangia of *R. subangulosum* have an angular appearance because of the formation of several papillae, whereas those of *R. oscillatoriae* have a large single apical papilla. Since no mature sporangia were found in the Frains Lake material a further comparison between those of the present parasite and the two previously mentioned species cannot be made, inasmuch as all three are spherical at first. Another comparison which is dependent upon the observations of mature material is concerned with the rhizoidal system. The rhizoids of *R. oscillatoriae* are delicate and at maturity form an extramatrical stalk upon which the sporangium rests. Only when more material of all stages in the life cycle of the present organism are found will its relationships be known with certainty.

SPECIES 6. (FIG. 13) Because of the absence of knowledge concerning the zoospore discharge, this fungus, which was found to be parasitic on *Ulothrix* sp. from the Huron River, Washtenaw County, Michigan, in January, 1955, cannot be identified with certainty.

The sporangia are sessile, spherical, 6 to 8 μ in diameter and are formed by enlargement of zoospore bodies which have encysted on the host cell. Projecting from the sporangium and penetrating the algal cell is a single outgrowth with a few delicate rhizoids which arise terminally (FIG. 13). These structures enter the host chloroplast, turning

it brown. Empty sporangia which possessed a lateral discharge pore were observed. The parasite most closely resembles *Rhizophydium laterale* (Braun) Rabenhorst (**1868**) in its sessile spherical sporangium, its zoospore discharge through a lateral pore, and its parasitism on members of the genus *Ulothrix*. It differs from this species in at least two structural features. The sporangial wall in the present fungus is thin and delicate and the rhizoidal tube is long, whereas, in *R. laterale*, the sporangial wall is 1.7 μ thick and the rhizoid is a short tube which is only two to three times longer than broad.

SPECIES 7. (FIGS. 15, 16) Parasite on *Melosira* sp. Of the several species of *Rhizophydium* and *Chytridium* described as parasitizing species of *Melosira*, this fungus from Michigan most closely resembles *R. melosirae* Friedman (**1952**), described from Austria. The American chytrid was found in Portage Lake, Washtenaw County, in November, 1954, and in March, 1955.

It resembles *R. melosirae* in its spherical sporangium, which was 8.5 to 10.5 μ in the Michigan material, its short extramatrical stalk upon which the sporangium rests, its branched rhizoidal system, and the fact that it parasitizes a species of *Melosira*. Only information on the manner of zoospore discharge will show, however, the true relationships of the present species. If it can be shown that sporangial dehiscence is by complete deliquescence of the zoosporangial wall, as in *R. melosirae*, then it can be assigned to this species with confidence.

PARASITE OF STEPHANODISCUS SP. (FIGS. 7–9)

A chytrid with an interesting method of zoospore discharge was found parasitizing the centric diatom *Stephanodiscus* sp. in Portage Lake, Washtenaw County, in November, 1954. Because of scarcity of material and (as a consequence) scanty observations, full knowledge of the rhizoidal system is not complete. Sporangial dehiscence was observed and found to take place by the deliquescence of a large portion of the zoosporangial wall (FIGS. 8, 9). The sessile sporangium, which lies tilted on the host cell, is broadly fusiform with an acute apex and a rounded knob-like basal portion. The latter is reminiscent of that found in *Podochytrium emmanuelense* (Sparrow) Sparrow and Paterson (**1955**) and to a less degree of *Rhizophydium clinopus* Scherffel (**1930**). In these fungi the zoospore cyst remains unexpanded to form the spherical basal portion of the sporangium. Although various clearing agents were used, the contents of the host cell remained so dense as to obscure observations on the nature of the complete rhizoidal system. The proximal portion was, however, visible and found to be a coarse

tube, 1.5 μ in diameter, arising from the base of the sporangium (FIG. 7).

The most striking feature of the present chytrid is the manner in which the zoospores are liberated (FIGS. 8, 9). The zoosporangium at first contains numerous droplets which gradually coalesce at maturity to form the oil globules of the zoospores. Subsequently, the spores are delimited, followed by the deliquescence of a portion of the wall just above the equator of the sporangium and the extrusion of the spores into a vesicle. They then oscillate for a few seconds and upon dissolution of the vesicle swim away free in the medium. They are spherical, 2.5 to 3 μ in diameter, with an eccentric oil globule and a posteriorly directed flagellum, 23 μ long.

It is hoped that this fungus will be found in greater numbers so that a more detailed study can be made of it, particularly with respect to the nature of the rhizoidal system.

DANGEARDIA SP. (FIGS. 23, 24)

This organism was found to be parasitic on the colonial green alga *Eudorina elegans* Ehr. from Portage Lake, Washtenaw County, in November, 1954.

The developmental pattern of the parasite is that typical for species of *Dangeardia*. The encysted zoospore on the surface of the mucilaginous algal sheath sends a fine germ tube into the thick gelatinous envelope where it continues growth until it reaches a host cell (FIG. 23). Following entrance of the germ tube into the algal cell there is a shrinking of the host cell and its contents turn brown (FIG. 24). The tenuous penetration tube in the gelatinous matrix of the alga gradually broadens until it is almost cylindrical. It continues to enlarge and subsequently forms a flask-shaped sporangium 8 to 10 μ in diameter by 15 to 20.5 μ high. Its protoplasm is at first granular, interspersed with refractive droplets which eventually coalesce to form numerous oil globules of uniform size and distribution (FIG. 24). Inasmuch as further developmental stages of the sporangium leading up to the delimitation of the zoospores and their eventual discharge were not observed, it could not be determined whether the sporangium was operculate or inoperculate. Empty, partially collapsed zoosporangia were found, however, with no indication of an operculum (FIG. 23).

This fungus most closely resembles two species of *Dangeardia*, namely *D. mammillata* Schröder (**1898**) and *D. laevis* Sparrow and Barr (**1955**), in the size, shape and development of the zoosporangium. Since its resting spore has not been observed it is not possible to ascer-

tain to which of the two species it belongs. *D. mammillata* has a spiny-walled resting spore and is formed after the conjugation of immature thalli, whereas in *D. laevis* it is smooth-walled and is not, so far as is known, formed after a sexual process. The Portage Lake fungus also resembles *D. ovata* (FIGS. 1–6) in the development of the zoosporangium. The two differ, however, in the shape of the sporangium, which is flask-shaped in the present species and ovoid in *D. ovata*.

? DANGEARDIA SP. (FIG. 26)

Among several other interesting chytridiaceous parasites found in the plankton from Crooked Lake in Southern Michigan in April, 1955, is an organism parasitizing *Hyalotheca* sp. which resembles a species of *Dangeardia* in its developmental pattern and a species of *Phlyctochytrium* in the possession of an endobiotic subsporangial swelling.

The zoosporangium is formed within the gelatinous sheath of the host, indicating a development similar to that of *Dangeardia* in which the germ tube broadens to form the sporangium. At maturity the latter is ovate to ellipsoidal, 17.5 to 18 μ high by 12 to 13 μ in diameter, its long axis being parallel to that of the host filament. From the endobiotic apophysis, which is 12 μ long by 5 μ wide, arise one or two broad rhizoids, 2 to 3 μ in diameter, which branch distally. The rhizoidal system ramifies through several host cells, causing their contents to shrivel and turn brown. The protoplasm of the sporangium is at first granular, with small scattered oil droplets, which coalesce and form at maturity the like-sized, uniformly spaced globules of the zoospores. Following cleavage the spores are liberated *en masse* through a single apical sporangial pore (FIG. 26). Subsequently, they remain clustered at the discharge pore and after a few seconds begin to oscillate and then swim away. They are 3.5 to 4.5 μ in diameter, posteriorly uniflagellate, with an eccentric oil globule 1.5 μ in diameter.

The disposition of this organism is difficult, since its developmental stages are not completely known. The manner in which the host cell is penetrated and subsequent method of zoosporangial formation within the gelatinous sheath must be observed in order to determine the chytrid's precise taxonomic position. Except for the fact that the sporangium is within the gelatinous matrix of the host, it resembles *Phlyctochytrium laterale* Sparrow (**1936**) in its subspherical sporangium and stout, branched rhizoidal system, which arises laterally from an endobiotic subsporangial apophysis. However, because of the position of the sporangium in the *Hyalotheca* sheath it is difficult to conceive of a type of development like that found in a species of *Phlyctochytrium* in which the

zoospore cyst itself enlarges into a sporangium. It is more reasonable to assume a sequence of zoosporangial development such as found in *Dangeardia* in which the germ tube broadens to form the reproductive rudiment. Hence the fungus is tentatively considered under that genus.

PARASITE OF BOTRYOCOCCUS BRAUNII Kütz. (FIG. 25)

During an investigation of chytridiaceous parasites in October, 1954, at Frains Lake near Ann Arbor, an interesting parasite of the green alga *Botryococcus braunii* Kütz. was found. Later, in May, 1955, the same fungus appeared in the plankton from Whitmore Lake, Livingston and Washtenaw Counties, Michigan. Its generic relationships cannot be precisely established at this time, for reasons indicated below.

Since *B. braunii* is a colonial alga with densely crowded cells, certain developmental features of the parasite of taxonomic importance were obscured. It was possible to determine, however, that the zoospore cyst, which is adherent to the gelatinous sheath of the host, produces a germ tube which penetrates the alga and enlarges into an apophysis. Because of the aforementioned structure of the host colony, observations on the apophysis and rhizoidal system were difficult. Attempts were made to clear the host with lactophenol, acetocarmine, and chloral hydrate. The latter proved to be the most satisfactory, and with subsequent crushing it was possible to observe the apophysis. Whether the germ tube entered the host cell proper and the apophysis formed there or whether it was formed wholly within the gelatinous sheath could not be determined, since the young stages were completely destroyed by the chloral hydrate. Although the clearing process was never complete there were some indications that rhizoids were produced, since there were discontinuities in the apophysis wall basally or laterally. At the points of these breaks there appeared to arise projections resembling the proximal portions of rhizoids. A short distance from the apophysis these projections appeared to fade into the host material.

In the development of the reproductive rudiment, the zoospore cyst expands into a cylindrical structure, 30 to 39 μ long by 12 to 19 μ in diameter, which is rounded at both apex and base. Its contents are at first granular, with small interspersed refractive droplets which coalesce at maturity to form the oil globules of the zoospores. The ensuing delimitation is followed by their discharge through an apical pore, at the mouth of which they remain motionless for a few seconds *en masse* before they begin to oscillate and finally to swim away. Each of the zoospores, which are 8 to 9 μ in diameter, contains an eccentric oil

globule 3 to 4 μ in diameter and bears a posteriorly directed flagellum 34 μ in length (FIG. 25).

This organism most closely resembles a member of the genus *Phlyctochytrium* in its inoperculate discharge through a single apical pore and its endobiotic apophysis, if the latter structure can be considered to be truly endobiotic. If, however, the apophysis is epibiotic it may have a closer affinity with members of the genus *Rhizoclosmatium*. Whether or not there are rhizoids is still open to question, although there is strong evidence for their presence in the aforementioned "projections" arising from the apophysis. It is possible that more of the extramatrical structures of the present parasite might be shown by the use of more delicate clearing techniques. Until such time as these are seen the precise taxonomic position of this fungus cannot be ascertained.

PARASITE OF MICROCYSTIS SP. AND ITS HYPERPARASITE (FIGS. 10, 11, 12)

Of the numerous fungi found in this investigation none is more puzzling in its relationships than a parasite found on *Microcystis* sp. It occurred in plankton from Whitmore Lake, Washtenaw and Livingston Counties, Michigan, during September 1954, and from Silver Lake, Washtenaw County, Michigan, in November 1955. Its development is similar to that of species of *Dangeardia*. The encysted zoospore resting on the gelatinous sheath of the host sends a delicate germ tube into the mucilaginous matrix (FIG. 10). The tube then enlarges to form a flask-shaped zoosporangium, 11.5 to 14 μ long by 6 to 8.5 μ in diameter The upper portion of this reproductive rudiment remains unchanged in width and eventually forms the neck of the sporangium, which is terminated by the thicker-walled zoospore cyst. It differs from species of *Dangeardia*, however, in its rhizoidal system. In the present organism the distal half of the penetration tube expands only slightly and forms an epibiotic rhizoidal axis. The mature sporangium therefore is not sessile on the host cell as in species of *Dangeardia*. Furthermore, this rhizoid does not penetrate a single algal cell but branches to form an interbiotic system the tips of whose numerous rhizoids penetrate several host cells. In this respect the fungus resembles a member of the Rhizidiaceae rather than the Phlyctidiaceae to which *Dangeardia* belongs. When more information is obtained, particularly with respect to zoospore discharge and nature of the resting spore, its precise taxonomic relationships can be ascertained. A new genus should probably be erected for it.

The hyperparasite of *Microcystis* sp. bears some resemblance to the

chytrid infecting *Chytriomyces*, *Phlyctidium mycetophagum* Karling (**1946**) in the shape of the sporangia, although in Karling's species they are more distinctly obpyriform than those of the Michigan fungus, which are broadly ellipsoidal. *Phlyctidium mycetophagum* is very different in the nature of its rhizoidal system. It has a single thread-like unbranched rhizoid whereas in the present fungus there is a much branched rhizoidal system. It is hoped that more material will be obtained of the Michigan chytrid in order to gain knowledge of its complete life cycle, which will show its precise taxonomic relationships.

SAPROLEGNEACEOUS PARASITES OF PHYTOPLANKTERS

Ectrogella bacillariacearum Zopf (**1884**) was found to be infecting *Synedra* sp. from Portage Lake in Washtenaw County, Michigan, during December, 1954. Although no zoospore discharge was observed, other structural features were so distinct as to identify it with certainty. In addition to the tubular sporangium, several empty cysts each containing a small pore were observed, indicating that discharge of the secondary zoospores had occurred.

Lagenidium rabenhorstii Zopf (**1878**) was found in plankton from Fleming Creek, Washtenaw County, Michigan, during February, 1954. It occurred abundantly on *Spirogyra* sp.

PHYCOMYCETOUS PARASITES OF ZOOPLANKTERS

Olpidium gregarium (Nowakowski) Schroeter (**1885**) was found in abundance in rotifer eggs from Frains Lake, Washtenaw County, Michigan, during April, 1954.

A fungus believed to be *Myzocytium zoophthorum* Sparrow was found infecting the adult rotifer *Notholca longispina* Kellicott in plankton from Silver Lake, Washtenaw County, Michigan, in the spring of 1954. Although the organism was fairly abundant, only the immature stages were found. The thallus was observed to be an irregular, lobulate, tube-like structure, 14 to 21 μ in diameter, whose contents resemble the characteristically pale gleaming aspect of members of the order Lagenidiales. It was similar to *Myzocytium zoophthorum* Sparrow (**1936**) in the size and shape of the thallus and in parasitizing members of the Rotifera. Until such time as the complete developmental stages and especially its non-sexual reproduction are observed, however, its precise relationships cannot be determined.

During the fall of 1953, there was found in northern Michigan, from Lake Nettie, Presque Isle County, a parasite of an adult rotifer belong-

ing to the genus *Polyarthra*. Information obtained was insufficient to indicate any precise relationship between the present organism and other species of Phycomycetes. The thallus was a holocarpic, endobiotic structure composed of a spherical sporangium 40 to 50 μ in diameter, and a long discharge tube 90 to 100 μ long by 7 to 11 μ in diameter. Sporangial dehiscence was inoperculate and the spherical spores, which were 5 to 6 μ in diameter with several refractive granules interspersed with small oil globules, appeared to ooze out of the sporangium, often in an amoeboid fashion, and to encyst at the orifice of the discharge tube as in *Aphanomyces* (FIG. 27).

The parasite most closely resembles *Pythiella vernalis* Couch (**1935**) in the possession of a spherical sporangium without mycelium or rhizoids and a long discharge tube. It differs from *P. vernalis*, however, in the fact that it infects a rotifer, whereas Couch's species is parasitic in hyphae of species of *Pythium*. It also resembles species of *Ectrogella* in its formation of an endobiotic, holocarpic unbranched thallus and its apparent diplanetism, in which the primary zoospores encyst at the tip of the discharge tube. It differs, however, in that the motility of the primary zoospores of the present fungus is suppressed, whereas in members of the genus *Ectrogella* they are distinctly biflagellate and motile. It is hoped that more material will be found of the present species in order to ascertain its precise relationships.

SUMMARY

One new species is described from a lake in Washtenaw County, Michigan, namely *Dangeardia ovata* n. sp., parasitic on *Sphaerocystis schroeteri* Chod. The parasitism by *Rhizophydium megarrhizum* Sparrow of *Lyngbya* sp. and *Aphanizomenon flos-aquae* (L.) Ralfs is discussed. Several incompletely known chytrids and two saprolegniaceous fungi parasitic on phytoplankters are presented. In addition, one chytridiaceous and two saprolegniaceous parasites found on zooplankters are discussed.

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NEW SPECIES OF GALERINA

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During the interval since our last contribution appeared (Mycologia **47**: 557–596. 1955), additional undescribed species of *Galerina* have come to light in both the flora of South America and that of North America. In this continuation of our field explorations we take great pleasure in acknowledging the financial assistance received from the National Science Foundation, Washington, D. C., and the Faculty Research Fund of the University of Michigan.

The fruiting pattern of *Galerina* continues to be sporadic, and, for most of the species, less predictable than one would expect. However, as more collections accumulate it appears that careful study of the restriction of certain species to mosses in certain genera, such as *Polytrichum*, may be worth while. We have hesitated to place much emphasis on this feature at present because of certain tendencies among lignicolous species which indicate lack of specialization as to substrate, some species occurring both on wood of hardwoods and conifers, though they are usually more abundant on one than the other.

The present paper contains 20 previously undescribed species, 3 varieties and one form previously undescribed. These taxa will all be treated in more detail in our forthcoming monograph. Color terms within quotation marks are taken from R. Ridgway, Color Standards and Color Nomenclature, Washington, D. C., 1912. It is apparent to us now, however, that the color of the various parts of the fruiting body is important taxonomically only when it deviates from a standard pattern. The standard pattern is an ochraceous-tawny to tawny cap, buff to yellow gills when young, and a honey-colored stipe. This pattern holds quite well for specimens from all areas from which we have collected as well as from those from which we have received material for identification. Consequently, for most species, matching plates in a color chart with colors of the parts of a fruiting body is by no means as essential as it is in the closely related genus *Cortinarius*.

[1] Papers from the University of Michigan Herbarium and the Department of Botany, No. 1073, University of Michigan, Ann Arbor, Michigan, and the Instituto Miguel Lillo, Tucumán, Argentina.

1. **Galerina borealis** sp. n.

Pileo 8-15 mm lato, obtuso-conico, pallide ochraceo-brunneo, hygrophano, in statu sicco pallide brunneolo, in exsiccatis griseolo-brunneo; lamellis latis, adnatis, subdistantibus, pallide alutaceis, dein pallide cinnamomeis; stipite 30-50 × c. 1 mm, uniformiter melleo vel in vetustis ad basin subobscuriore, velo in juvenilibus e nonnullis fibrillis sparsis consistente, apice pruinoso. Sporis 9–11.5 × 5–6 μ, i.e., pro ratione magnis, pallide ochraceis (KOH), lineaque levi zonam suprahilarem levem delimitante destitutis; basidiis tetrasporis; cheilocystidiis *26–32* × *3–7* μ apice *2–3* μ crassis et frequenter capitatis (capitulis 3–5 μ in diam.), hyalinis; epicute pilei haud gelatinosa; fibulis praesentibus.

Pileus 8–15 mm broad, campanulate becoming obtusely conic or with a flaring margin, surface glabrous, moist, hygrophanous, striate when moist, pale ochraceous-tawny fading to pallid brownish and drying a *grayish* brown; odor none.

Lamellae broad, adnate, subdistant, pallid buff becoming pale dull cinnamon, edges even.

Stipe 3–5 cm long, cc. 1 mm thick, equal, fragile, honey-color over all or in age the base only slightly darker, at first with a few scattered fibrils from the rudimentary veil, in age naked except for the pruinose apex.

Spores 9–11.5 × 5–6 μ, inequilateral in side view, ovate in face view, *pale ochraceous* in KOH, smooth (hence no plage), apical callus present; basidia 4-spored; pleurocystidia none; cheilocystidia *26–32* × *3–7* × *2–3* × *3–5* μ,[2] filamentose-capitate to merely filamentose or some more or less ventricose at base, hyaline in KOH; gill trama yellowish in KOH, regular; pileus trama with a cuticle of narrow (4–8 μ), more or less incrusted non-gelatinous hyphae over a highly colored hypoderm; clamp connections present.

Habit, habitat and distribution: Gregarious on moss, Granite Creek, near Nordman, Idaho, Oct. 15, 1956, *Sm-54772*—TYPE.

In the characters of the cheilocystidia it is related to *G. pistillicystis* and the group centering around *G. sideroides* but is distinct on the basis of the large pale spores. The dried specimens are duller in color than in most species of *Galerina*.

2. **Galerina californica** sp. n.

Species characteribus stirpis *Hypnorum* sporisque parvulis (6.5–8(–9) × 4–4.5 μ) insignis; annulo nullo.

Pileus 4–6 mm broad, obtusely conic to convex, becoming broadly convex with a spreading margin, surface moist and hygrophanous, at

[2] Measurements give length first, diameter of basal enlargement, diameter of neck, and finally diameter at apex.

first with scattered buff fibrils over margin, striate to the disc, cinnamon on disc and ochraceous-tawny on margin; odor and taste not recorded.

Lamellae close, broad, adnate, pinkish buff becoming "cinnamon," edges even and in some pallid.

Stipe 1.5–2 cm long, cc. 0.5 mm thick, equal, crowded, bay-brown at base, concolor with gills at apex, at first with a thin coating of fibrils or with merely scattered buff fibrils from a rudimentary veil, apex pruinose.

Spores 6.5–8(–9) × 4–4.5 μ, ovate in face view, somewhat inequilateral in side view, *cinnamon* in KOH, surface slightly roughened and plage boundary a faint line, no separations of outer wall noted; basidia 4-spored; pleurocystidia none; cheilocystidia 40–60 × 3–7 μ, narrowly fusoid, hyaline or walls faintly yellow over basal area, thin-walled, apices acute; gill trama regular, cinnamon in KOH; pileus trama dark cinnamon in KOH, hyphae not encrusted or only slightly so; clamp connections present.

Habit, habitat and distribution: Cespitose on soil in a trail under redwoods, Trinidad, California, Dec. 4, 1956, *Sm-56299*—TYPE.

This species is closest to *G. subbadia* but the boundary of the plage is less distinct, no pilocystidia were found, a pale buff veil was present, and no capitate cheilocystidia were seen. It is close to *G. humicola* but that has smaller spores, subcapitate cheilocystidia, a whitish veil and a sharply conic pileus.

3. **Galerina castanescens** sp. n.

Species stirpis *Sideroidis* atque a *G. pseudobadipede* Joss. coloribus obscurioribus nec non a *G. agloea* nob. habitatione in lignis coniferis et cheilocystidiis capitatis distinguenda.

Pileus 7–16 mm broad, obtusely-conic, expanding to campanulate or expanded-umbonate, surface glabrous, moist and hygrophanous, rusty to tawny when fresh, scarcely paler when dried, margin at times fringed with a few fibrils; odor and taste not recorded.

Lamellae close, narrow, narrowly adnate, dull ochraceous tawny, edges even.

Stipe 2.5–4(–5) cm long, 1–2 mm thick, honey-color above, soon *dark rust-brown* below and darkening to red-brown over all in drying, veil fairly well developed, grayish-pallid, leaving a faint superior zone or only with scattered fibrils above.

Spores 7–9 × 4–4.5 μ, smooth, narrowly ovate to elliptic in face view, obscurely inequilateral in side view, near "ochraceous tawny" in KOH; basidia 2- and 4-spored; pleurocystidia none; cheilocystidia abundant, ventricose at base (8–12 μ), neck 2–3 μ and apex acute to capitate, capitellum 4–5 μ, overall length 20–28 μ, hyaline in KOH, thin-walled and yellowish; gill trama subregular, central strand more or less cinnamon

in KOH, subhymenium gelatinous and yellowish-hyaline; pileus trama homogeneous and cinnamon in KOH, cuticle of radial nongelatinous hyphae 4–7 μ in diam.; clamps present.

Habit, habitat and distribution: Gregarious on conifer logs, Granite Creek, Nordman, Idaho, Oct. 9, 1956, *Sm-54347*—TYPE.

The specimens have dried cinnamon color with the stipes a darker red-brown. The lack of a distinct gelatinous pellicle separates it from *G. stylifera* and related species, and the darker colors from *G. pseudobadipes*.

4. **Galerina castaneipes** sp. n.

Pileo 8–16 mm lato, viscido, ochraceo-brunneo, lamellis confertis, latis; stipite 10–20 × 1.5–2 mm, glabro, obscure ferrugineo-brunneo deorsum atque melleo sursum; sporis 7.5–9.5(–10) × 4–5.5 μ, adhaerenter verrucoso-rugosis, disco levi suprahilari bene evoluto, callo prominente ornamentatione destituto instructis; basidiis 4-sporis; pleurocystidiis raris vel sparsis, 50–70 × 9–14 μ, tenuitunicatis, fusoideoventricosis, apice attenuato elongato praeditis, sursum obtusis, interdum apice breviter lateraliter appendiculatis; hyphis epicuticularibus hyalinis, gelatinosis angustis (3–6 mm); fibulis praesentibus.

Pileus 8–16 mm broad, convex to broadly convex expanding to plane, surface glabrous, viscid, ochraceous-tawny moist, fading to near cinnamon-buff or with more yellow; odor and taste not recorded.

Lamellae close, broad, horizontal, bluntly adnate or with a decurrent tooth, near "buckthorn brown" at maturity, edges even or nearly so.

Stipe 1–2 cm long, 1.5–2 mm thick, equal down to a slightly bulbous mycelial base, *surface glabrous*, dark rusty brown below, honey-color above, darkening over all in drying.

Spores 7.5–9.5(–10) × 4–5.5 μ, slightly ovate in face view, subelliptic in side view, ornamentation well developed, in the form of a warty-rugulose adherent layer, plage distinct and smooth, rusty brown (rather dark) in KOH, with a *prominent* callus (immature spores appearing to have an apical pore), *ornamentation often ending in a ragged line just short of spore-apex;* basidia 4-spored, hyaline, extending half their length when sporulating, 28–34 × 6–7 μ; pleurocystidia rare to scattered 50–70 × 9–14 μ; hyaline in KOH, thin-walled, fusoid-ventricose with long necks and obtuse apices, occasionally enlarged slightly below apex, rarely with one or more short lateral projections near apex; cheilocystidia similar to pleurocystidia (none seen with lateral projections near apex); gill trama of parallel hyphae, hyaline to yellowish in KOH; pileus trama yellow to fulvous (in KOH) and with ochraceous incrusting pigment on hyphae, pellicle well developed and of hyaline to yellowish gelatinous narrow (3–6 μ) hyphae; clamp connections present.

Habit, habitat and distribution: Subcespitose on rotting oak logs, Grants Pass, Oregon, Nov. 12, 1956, *Sm-55523*—TYPE.

The distinguishing field characters are the resemblance in appearance to *G. autumnalis* and the absence of a veil. The shape of the spores is the same as in *G. oregonensis* but the spores are larger and have a more distinct apical pore. The fungus definitely occurs on rotting wood and, of course, lacks a veil. We are not placing any taxonomic significance on the rare pleurocystidia which show short lateral protrusions, since there were so few of them, but we believe it is important to note their presence.

5. **Galerina cinnamomea** sp. n.

Pileo 10–20 mm lato, convexo, glabro, humido vel subviscido, cinnamomeo; lamellis latis, subdistantibus; stipite 30–35 × 1.5 mm, ad basin obscurissime cinnamomeo, sursum pallidiore, zona fibrillosa e velo orta apicali sed haud annuliformi ornato, dein evelato. Sporis 8.5–11 × 5.5–6.5 μ, verrucosis, exosporio laxe applicato, basidiis tetrasporis, flavis (KOH), pleurocystidiis raris vel sparsis, 40–60 × 9–14 μ, fusoideo-ventricosis, hyalinis, rarius flavidis, apice acutis vel subacutis frequenterque, flexuosis, 3–5 μ diam.; cheilocystidiis pleurocystidiis similimis; epicute pilei tenui, ex hyphis tenuibus (3–5 μ), gelatinosis efformata; hyphis fibulatis.

Pileus 1–2 cm broad, convex, glabrous, moist, to slightly viscid (pellicle thin), color evenly cinnamon and drying only slightly paler; odor and taste not recorded.

Lamellae broad, arcuate, subdistant, concolorous with the pileus.

Stipe 3–3.5 cm long, 1.5 mm thick, equal, surface very dark cinnamon below, paler above, reddish cinnamon over all when dried, with an apical or superior zone of fibrils which disappears in age.

Spores 8.5–11 × 5.5–6.5 μ, ovate in face view, somewhat inequilateral in side view, dark cinnamon in KOH, heavily ornamented but with a smooth plage, the surface rugulose-verrucose and enveloping the spore comparatively loosely (not calyptrate, however); basidia 4-spored, yellow in KOH; pleurocystidia rare to scattered, 40–60 × 9–14 μ, fusoid-ventricose, hyaline in KOH or content rarely yellowish, apices acute to subacute and neck often flexuous and only 3–5 μ wide near apex; cheilocystidia similar to the pleurocystidia, numerous; gill trama regular, ochraceous to tawny in KOH; pileus trama with a thin gelatinous pellicle of narrow (3–5 μ) ochraceous, incrusted hyphae or hyphae hyaline and smooth; clamp connections present.

Habit, habitat and distribution: Subcespitose on chip-dirt in a slashing, Grants Pass, Oregon, Nov. 10, 1956, *Sm-55422*—TYPE.

The generally cinnamon colors, large dark colored spores of the *G. rugisperma* type, thin gelatinous pellicle, and scattered acute to subacute pleurocystidia distinguish it as a species. It is close to *G. autumnalis* but lacks a membranous annulus and has more cinnamon colors.

6. **Galerina dicranorum** sp. n.

Species characteribus stirpis *Mycenopsis* gaudens et cheilocystidiis filamentosis vel filamentoso-subcapitatis (24–35 × 4–5 μ) insignis; habitu *G. hypnorum*.

Pileus 7–15 mm broad, conic to convex, surface glabrous, moist and hygrophanous, striate, margin straight when young, near ochraceous-tawny moist, fading from disc out to a bright yellow-brown.

Lamellae sinuate, distant at maturity, 2–3 mm broad, nearly concolorous with pileus dried, edges even or nearly so.

Stipe 4–5 cm long, 1.5–2 mm thick, hollow, cartilaginous, apex pruinose, dingy honey-color, scarcely darker below, base covered with a thin tomentum, veil none.

Spores 9–11 × 5–6 μ, elliptic to slightly oval in face view, in side view obscurely inequilateral, practically smooth and plage very faintly delimited, ochraceous-tawny in KOH, dark reddish-brown in Melzer's sol.; no distinct apical pore present; basidia 4-spored; pleurocystidia none; cheilocystidia 24–35 × 4–5 × 6–8 μ, filamentous to filamentous-subcapitate, in some the basal part slightly ventricose but head usually the broadest part, hyaline, smooth, thin-walled; gill trama interwoven, yellowish in KOH; pileus trama with a thin pellicle of narrow (3–5 μ) hyphae, laticiferous hyphae present and dark red brown in Melzer's sol., pellicle not gelatinous, color of hyphae pale ochraceous and clamp connections present at the cross walls.

Habit, habitat and distribution: On a species of *Dicranum*, gregarious, south of Sinclair, Maine, Aug. 27, 1956, *Bigelow 4421*—TYPE, *4144, 4592, 4648, 4719*.

This species appears to be close to *G. vexans* but differs sharply in the characters of the cheilocystidia. In revived material the pellicle appeared slightly translucent, which would indicate that the cap when fresh was slightly lubricous. The filamentose subcapitate cheilocystidia might cause one to seek for this species in the stirps *Sideroides*, but it is not related there by its other characters. Bigelow 4144 had scattered fibrils on the stipe in the largest specimen, but the collector stated that no veil was present. These specimens are about the statue of *G. hypnorum*.

7. **Galerina filiformis** sp. n.

Pileo 2.5–4 mm lato, 4.5 mm alto, anguste conico, humido, cinnamomeo-alutaceo; lamellis angustis, distantibus, ascendentibus; stipite 20–25 × 0.5 mm, sursum pallido leniter farinoso, deorsum nudo; basidiis tetrasporis; sporis 8–11 × 5–6 μ, multis calyptratis, superficie sub perisporio levi vel lenissime subrugulosa; pleurocystidiis sparsis, clavatis vel vesiculosis, 26–34 × 12–20 μ; hyphis fibulatis.

Pileus 2.5–4 mm broad and 4.5 mm high, narrowly conic, surface moist, hygrophanous, margin connivent when young, soon straight and

sterile, color cinnamon-buff to "ochraceous-tawny" and striate; flesh soft and delicate, odor none.

Lamellae narrow (linear), not extending to the cap margin, *distant*, ascending and only touching the apex of the stipe, pinkish buff, edges even.

Stipe 2–2.5 cm long, 0.5 mm thick, pallid above and faintly pruinose, cinnamon-buff downward, naked over lower part.

Spores 8–11 × 5–6 μ (4-spored) or 11–14 × 7–8.5 μ (1- or 2-spored), ovate in face view, somewhat inequilateral in side view, a fair percentage distinctly calyptrate, plage smooth and distinctly bordered, remainder of wall smooth to slightly wrinkled, ochraceous tawny in KOH or a little darker; basidia 4-spored (2- and 1-spored also); pleurocystidia scattered, clavate to vesiculose, 26–34 × 12–20 μ, thin-walled, some present which are similar to cheilocystidia (1-spored basidia may simulate pleurocystidia), cheilocystidia variable, 30–50 × 4–12 μ, neck 2–5 μ, apices enlarged in some and 4–5 μ in diam., some cylindric-capitate, some ventricose at the base and with flexuous neck and enlarged apex, also some clavate cells up to 18 μ in diam. present; gill trama ochraceous; pileus trama with a thin non-gelatinous pellicle; clamps present.

Habit, habitat and distribution: On a mossy redwood log, Trinidad, Calif., Dec. 10, 1956, *Sm-56556*—TYPE.

This is the only species, to our knowledge, to show both pleurocystidia and a fair percentage of calyptrate spores. The stature is that of *G. subfiliformis*.

8. **Galerina anelligera** sp. nov.

Species characteribus subsectionis *Calyptrosporae* gaudens et *G. psathyrelloidi* proxima sed sporis minoribus (9–12.5 × 5–6.5 μ), pileo minore (6–9 mm lato) et stipite breviore (10 mm longo), nec non veli abundantia qua ex re annulus fibrillosus formatur, bene distinguitur.

Pileus 6–9 mm broad, obtusely conic becoming conic-campanulate, surface glabrous, moist, hygrophanous, dull ochraceous tawny fading to a dingy cinnamon buff; odor and taste not distinctive.

Lamellae broad, close, adnate, concolorous with the pileus.

Stipe 1 cm long, 2 mm thick, equal, heavily pruinose above and sheathed below up to the fibrillose annulus with pale buff (yellowish) fibrils, dark brown over all beneath the pruina and fibrils.

Spores 9–12.5 × 5–6.5 μ, ovate in face view, somewhat inequilateral in side view, nearly "cinnamon-brown" in KOH, calyptrate, plage smooth, remainder of surface faintly roughened (under oil); basidia 4-spored; pleurocystidia none; cheilocystidia abundant, 40–55 × 7–11 μ, fusoid-ventricose, necks 4–5 μ thick and apices obtuse to slightly enlarged, hyaline to yellowish in KOH; gill and pileus trama well colored

(rusty) from incrusting pigment; cuticle a layer of radial narrow non-gelatinous hyphae, the walls encrusted; clamp connections present.

Habit, habitat and distribution: On wet duff under conifers, South Fork of Lake Fork, Payette Lakes, Idaho, Sept. 17, 1956, *R. Shaffer* (Sm-53494)—TYPE.

The buff colored veil and dull brown calyptrate spores relate this collection to *G. psathyrelloides*. It differs in smaller spores, well-developed veil, and heavy pruinosity over apical region of the stipe. It has the appearance of a *Psathyrella* but the spores clearly indicate its position in *Galerina*.

9. **Galerina leucobryicola** sp. nov.

Species characteribus stirpis *Mycenopsis* gaudens et sporis parvulis (7–9 × 4.5–5.5 μ) insignis; stipite unicolori, velo tenui, hyphis tramatis pilei abundanter pigmento incrustatis.

Pileus 6–12 mm broad at base, obtusely conic to convex, broadly convex in age, margin straight in youth, surface glabrous, moist, hygrophanous, translucent striate and ochraceous tawny to tawny at first, near pinkish buff faded, flesh thin, concolorous with surface, fragile, odor and taste not recorded.

Lamellae distant, moderately broad, broadly adnate, ochroleucous young, tawny in age from the spores, edges white fimbriate.

Stipe 2–3 cm long, up to 1 mm thick, equal, pallid honey-color over all and with a few pallid fibrils from the remains of a thin veil, apex pruinose from caulocystidia.

Spores 7–9 × 4.5–5.5 μ, ovate in face view, inequilateral in side view, smooth or very minutely marbled, plage not delimited or only very faintly so in a few spores, tawny in KOH, *deep reddish brown* (pseudo-amyloid) in Melzer's; basidia 4-spored 22–25× 7–8 μ, hyaline but many basidioles present with yellowish-cinnamon content in KOH; pleurocystidia none; cheilocystidia 30–40(–50) × 7–10 × 6–7 μ, base slightly ventricose, neck 5–6 μ thick and apex obtuse to subcapitate and 6–7 μ, thin-walled, hyaline or with a tinge of cinnamon in the wall at the base; gill trama heavily incrusted with ochraceous pigment; pileus trama with a thin non-gelatinous pellicle and beneath it a hypoderm consisting of heavily incrusted (tawny to rubiginous in KOH) hyphae bearing clamps at the cross walls.

Habit, habitat and distribution: Gregarious on *Leucobryum glaucum*, Pinckney Recreation Area, Washtenaw Co., Michigan, May 29, 1955, *Sm-49533*—TYPE.

The smooth to practically smooth spores which give a strong pseudo-amyloid reaction, the evenly colored stipe and thin veil, and the heavy

pigment incrustations of the hyphae of the pileus trama are distinctive. It is close to *G. naucorioides* but has smaller spores, a paler stipe, a straight pileus-margin and a very different habitat. The fruiting bodies were definitely attached to the moss gametophytes.

10. **Galerina longinqua** sp. n.

Pileo aurantio-brunneo, in vetustis breviter striatulo, conico-obtuso dein expanso-umbonato, usque ad 15 mm lato. Lamellis concoloribus, distantibus, adnatis vel adnexis. Stipite concolori, basin versus atrobrunneo, usque ad 50 × 3 mm, apice extremo pruinato; velo nullo; sporis verruculoso-punctatis, supra hilum levibus, 10–13 × 6.8–7.8 μ; basidiis bisporis; cheilocystidiis plerumque ampullaceis et saepe subcapitatis, 38–55 × 5.5–11 μ; pleurocystidiis typicis rarissimis; epicute pilei perlaxa, hyphis hyalinis tenuissimis saepe undulatis; dermatocystidiis in pileo nullis, in stipitis parte media absentibus. Hyphis omnibus fibulatis.

Pileus up to 15 mm broad, conic to convex when young, later flat-convex to concave with a rounded umbo; evelate, orange brown, thin fleshy, with the margin at first straight and smooth, later striate with short transparent striae.

Lamellae orange brown, distant, rather broad, adnate (especially when young) or adnexed and then often weakly sinuate.

Stipe up to 50 × 3 mm, orange brown at apex, gradually dark red brown below, cartilaginous, fistulose, without veil even in the youngest stages, the extreme apex pruinate when young.

Spores 10–13 × 6.8–7.8 μ, broadly almond-shaped, with attenuate, sometimes extended apex, rather well colored, with often very thick complex wall, the exosporial ornamentation rather regularly and finely verruculose-punctate, with a distinctly limited plage, with or without a suprahilar applanation, without depression; basidia 2-spored (rarely 1- or 3-spored), 27.5–28.8 × 7.3–9.7 μ; pleurocystidia few, scattered, like cheilocystidia; a few cystidioid bodies slightly thick-walled (1 μ); cheilocystidia numerous, ventricose at base (ampullaceous), and then with a long (25–30 μ) neck, or more rarely ventricose below or in middle and then somewhat pedicellate, the tip very frequently subcapitate, the capitellum not very striking and the difference between the diameter below it and its own diameter very small (e.g. constriction below 4–4.5 μ diam., capitellum 4.8 μ diam.), the neck usually attenuate toward the apex, less frequently filamentous-equal, about 4 μ thick, ventricose portion 5.5–11 μ thick, total length 38–55 μ hymenophoral trama regular, of very short and broad hyphal cells, hyphae parallel; pellicle of pileus well developed, hyaline, consisting of thin hyaline hyphae which are rather spaced and often wavy, indicating a gelatinous layer in the fresh material; pileocystidia none; caulocystidia absent at the middle of the stipe, but present at the apex and descending from gill edges down the apex of stipe; all hyphae with clamp connections.

On mosses, single or in small clusters, on hills north of Green Gorge, Macquarie Isld. (SE of Australia), March 29, 1954, coll. *J. Bunt*, TYPE (LIL).

11. **Galerina macquariensis** sp. n.

Pileo 10–25 mm lato, hebeter flavo, apparenter viscido, frequenter ruguloso, papillato vel umbonato, margine striato, convexo, dein centro applanato, lamellis alutaceis, subdistantibus, late adnatis; stipite 20–45 × 3–5 mm, pallide brunneo nec in parte basali obscuriore, annulo supero ornato; sporis 8.2–9.7 × 5.5–6.7 μ, calyptratis, plerumque ornamentatione paullum projiciente ferruginea verrucoso-maculosa exosporiali praeditis; basidiis tetrasporis; cystidiis ad aciem lateraque lamellarum, numerosis, ampullaceis, saepe appendiculis sterigmatoideis ornatis; epicute bene evoluta apparenterque gelatinosa; hyphis fibuligeris.

Pileus up to 25 mm broad, dull yellow, apparently viscid, often wrinkled, umbonate or papillate, with striate margin, convex, later flattened on middle portion.

Lamellae buff color, subdistant, broadly adnate, rather broad.

Stipe 20–45 × 3–5 mm, pale brown and not visibly darker below, cartilaginous, equal but at times with a tapering base, fistulose, with an apical annulus.

Spores 8.2–9.7 × 5.5–6.7 μ, ellipsoid or ellipsoid-ovoid, distinctly although slightly verruculose-rusty on pale ochraceous-melleous ground, this exosporial ornamentation sometimes missing, but always with a distinct and persistent perisporial covering offering exactly the aspect of the calyptrate spores of the *G. cerina* complex with its characteristic ears, with a very well marked plage, binucleate; basidia 4-spored, 20.5–23 × 6.5–8.3 μ, very rarely in some individuals a few 2-spored basidia present; pleurocystidia and cheilocystidia similar, scattered, hyaline to pale stramineous or brownish, 38–52 × 11–22 μ, always ampullaceous with more or less cylindrical neck 9.5–12 μ broad, apex obtuse or with up to four apical sterigmatoid but obtuse prongs, below always strongly ventricose; subhymenium not gelatinized or not distinctly so in the material at hand; hymenophoral trama consisting of subparallel hyphae, regular, rather pallid stramineous or melleous-ochraceous, without incrusting pigment or with very little, in adult specimens its elements usually remarkably voluminous; pellicle of the pileus well differentiated, hyaline or pale *stramineous*, with the uppermost layer showing signs of having been gelatinized although now rather dense except for the outermost hyphal ends, somewhat wavy hyphae with free ends and a diameter of only 1.5–1.7 μ numerous; hypodermium consisting of very voluminous and often short hyphal elements which are repent-appressed and parallel or subparallel with each other; clamp connections regularly present.

Habit, habitat and distribution: On peats of raised coast terrace among various mosses, but carpophores although attached to these never

seen attached to *Sphagnum*, Langdon Pt., West Point, and Half Moon Bay, Macquarie Is., fruiting in March and April, *J. Bunt* 1950–51 (LIL, MICH). The MICH collection is the HOLOTYPE.

The "white bloom" indicated by the collector on the stipe must refer to white fibrils from the veil below the annulus. There are no dermatocystidia at the middle of the stipe or on the pileus.

12. **Galerina mainsii** sp. n.

Pileo 6–12 mm lato, siennicolori; lamellis latis, distantibus, stipite 20–30 × 1–1.5 mm, apice pruinoso, nudo in parte inferiore; sporis manifeste asperulatis, late ovoideis, 9–11 × 6.5–7.5 μ (eis *G. latisporae* comparabilibus); basidiis bisporis; pleurocystidiis 50–65 × 10–14 μ, fusoideo-ventricosis, apice subacutis, membrana partis ventricosae usque ad pedicellum pallide cinnamomea (KOH), aut tenuitunicatis aut membrana leniter subincrassata instructis in pedicello tantum; epicute pilei haud gelatinosa; fibulis praesentibus.

Pileus 6–12 mm broad, obtusely conic to convex becoming broadly convex, glabrous, moist and hygrophanous, "Burnt Sienna" (orange-brown) moist, fading to yellowish on the disc first, striate when moist.

Lamellae broad, broadly adnate, distant, edges minutely fimbriate, yellowish when young and tawny at maturity.

Stipe 2–3 cm long, 1–1.5 mm thick, equal, evenly pale reddish tawny throughout as dried, pruinose near apex from caulocystidia, naked over the lower two-thirds.

Spores 9–11 × 6.5–7.5 μ, *broadly* ovate in face view, broadly inequilateral in side view, distinctly roughened under oil and with a smooth plage, pale tawny in KOH, rubiginous in Melzer's (pseudo-amyloid), with a very slight callus; basidia 2-spored, 23–26 × 7–8 μ, in KOH hyaline but many basidioles with dull cinnamon content; pleurocystidia 50–65 × 10–14 μ, fusoid ventricose part *down to and including the pedicel pale cinnamon* in KOH, smooth, thin-walled or wall only very slightly thickened in the pedicle; cheilocystidia abundant, similar to pleurocystidia and with slightly thickened walls in the pedicel; gill trama of tawny encrusted hyphae; pellicle of pileus of appressed narrow non-gelatinous yellowish hyphae, tramal body of enlarged hyphae deep reddish tawny in KOH from pigment incrustations; clamp connections present.

Habit, habitat and distribution: Gregarious on moss, Logan Pass, Glacier National Park, Montana, *E. B. Mains 6116*, Aug. 20, 1941 —TYPE.

This species is not the two-spored form of *G. diabolissima* for a number of reasons: the plage is well formed, the colors are too vivid, the cystidia are too large, and the spores are too large (9–11 × 6.5–7.5 μ as compared to 7–8 × 4.2–4.7 μ) even allowing for the two-spored

condition. The lack of pileocystidia and any appreciable number of caulocystidia over the lower two-thirds of the stipe rules out both *G. vittaeformis* and *G. atkinsoniana*. Rather, it seems to be very close to *G. latispora* from which it differs in the colored cystidia, broad lamellae, and more strongly ornamented spores.

13. **Galerina nigripes** sp. nov.

Pileo 8–11 mm lato, obtuse conico vel campanulato, glabro, humido, hygrophano, brunneo sed inter strias ochraceo; lamellis latis, subdistantibus, ascendentibus, adnatis, pallide ochraceo-brunneis; stipite 23–30 × 1 mm, subnigro ad basin, pallide melleo ad apicem, in exsiccatis etiam basi atra praedito, nudo; sporis 8–11 × 5–5.5 μ, tenuitunicatis et multis in KOH collabentibus, pallide flavido-cinnamomeis in KOH, minute ornamentatis, iodi ope (reactione melzeriana) haud obscurius coloratis (inamyloideis); basidiis 4-sporis, cheilocystidiis abundantibus, 22–34 × 5–8 μ, tibiiformibus, capitulis 4–5 μ diam., epicute haud gelatinosa; hyphis haud fibulatis.

Pileus 8–11 mm broad, obtusely conic to campanulate, glabrous, moist, hygrophanous, tawny on disc and striae, ochraceous tawny between striae; odor and taste not recorded.

Lamellae broad, subdistant, ascending adnate, pale ochraceous-tawny.

Stipe 2.5–3 cm long, c. 1 mm thick, equal, *blackish at base*, pallid honey-color above, retaining the dark base when dried, naked (no sign of a veil).

Spores 8–11 × 5–5.5 μ, almost elliptic in face view, very thin-walled and many collapsing in KOH, pale yellow-cinnamon in KOH singly, pale cinnamon in groups, very minutely ornamented, *not darkening* in Melzer's sol.; basidia 4-spored; pleurocystidia none; cheilocystidia abundant, 22–34 × 5–8 × 2–3 × 4–5 μ; tibiiform, hyaline, smooth, thin-walled; gill trama regular, yellowish in KOH; pileus trama yellowish in KOH, hyphae slightly incrusted; pellicle of pileus thin, of narrow, appressed hyphae with non-gelatinous walls; clamp connections absent.

Habit, habitat and distribution: Gregarious on a mossy oak trunk, McLeod (near Grants Pass), Oregon, Nov. 15, 1956, *Sm-55691*—TYPE.

The blackening base of the stipe distinguishes this species from *G. graminea* and *G. brunneimarginata*, both of which grow in quite different habitats.

14. **Galerina nordmaniana** sp. n.

Pileo 8–13 mm lato, pallide cinnamomeo-brunneo; lamellis cinnamomeis, late adnatis, vel subdecurrentibus, latis, distantibus vel subdistantibus; stipite 20–23 × 1–5 mm, fragili, cinnamomeo-brunneo in parte inferiore, sursum pruinoso et melleo, nudo in maturis. Sporis 8.3–11 × 5–6 μ, obscure ferrugineo-brunneis in KOH, verrucoso-rugosis (disco levi suprahilari excepto), raro exosporio separibili circum discum levem; basidiis tetrasporis; pleurocystidiis sparsis, 30 70 × 9–14 μ, fusoideo-

ventricosis, apice acutis vel subacutis, hyalinis; cheilocystidiis plerumque angustioribus quam pleurocystidia sed ceterum simillimis; hyphis epicuticularibus pilei haud gelatinosis; hyphis omnibus fibulatis.

Pileus 8–15 mm broad, convex, margin straight, expanding to broadly convex, surface moist and hygrophanous, pale cinnamon-brown moist, fading to pinkish buff or warm buff (pale yellow), glabrous; flesh very soft, concolorous with the surface, odor none, taste mild.

Lamellae broadly adnate to subdecurrent, broad, distant or nearly so, "cinnamon," edges uneven and pallid.

Stipe 2–2.5 cm long, 1–5 mm thick, equal, fragile, near cinnamon-brown below and honey-color above, naked to the pruinose apex in age, all traces of rudimentary veil soon gone.

Spores 8.5–11 × 5–6 μ, almost russet in KOH (dark *dull* rusty brown) surface rather coarsely warty-wrinkled over all except the well defined smooth plage, rarely the outer wall separating slightly around the plage, callus present but inconspicuous; basidia 4-spored; pleurocystidia scattered, 50–70 × 9–14 μ, fusoid ventricose, the apices acute to subacute and often with a slight enlargement just back of the apex, walls thin and hyaline as revived in KOH, smooth, walls flexuous in some; cheilocystidia usually narrower than pleurocystidia but otherwise similar (no color seen in the walls); gill trama subregular, hyaline to ochraceous in KOH; pileus trama with a tawny yellow hypodermal zone, the cuticle of non-gelatinous narrower hyphae, color mostly located in the wall, pigment incrustations slight; clamp connections present.

Habit, habitat and distribution: On moss along a road bank, Granite Creek, near Nordman, Idaho, Oct. 9, 1956, *Sm-54260*—TYPE.

This seems to be intermediate between *Galerina saltensis* and *Galerina orcina*. The latter differs in having pleuro- and cheilocystidia with more rigid and often, over lower part, slightly colored walls, in the broader spores in relation to their length, and a less conspicuously darkening stipe. There is very likely a significant difference in the color of the gills also—"cinnamon" as compared with "ochraceous-tawny"—but more collections of *G. nordmaniana* are needed for comparison on this point, and spore deposits should be compared.

15. **Galerina payettensis** sp. nov.

Pileo 10–15 mm lato, conico-campanulato, umbone conico quali in *G. triscopa*, obtuso, cinnamomeo, hygrophano, in stato sicco cinnamomeo-alutaceo; lamellis latis, confertis; stipite 10–20 × 2 mm, brunneo in parte inferiore, pallidiore in apice conspicue pruinoso; sporis 9–11 × 5–6.5 μ, in 25 centisimis sporarum calyptratis, pallide brunneis, minutissime rugulosis vel levissimis; basidiis tetrasporis; cheilocystidiis qualibus in *G. cerina* var. *ampullicyste;* hyphis fibulatis.

Pileus 10–15 mm broad, conic-campanulate, the umbo conic as in *G. triscopa*, glabrous, moist, hygrophanous, "sayal brown" (dull cinnamon) fading to cinnamon buff.

Lamellae *broad*, close, adnate, dull ochraceous tawny.

Stipe 1–2 cm long, 2 mm thick, equal, dull brown below, paler above, apex dingy cinnamon buff and heavily pruinose, veil leaving a thin annular zone but no sheath below the zone.

Spores 9–11 × 5–6.5 μ, inequilateral in side view, ovate in face view, about 25% calyptrate, pale tawny in KOH, outer layer smooth to minutely wrinkled; basidia 4-spored; pleurocystidia none; cheilocystidia 30–46 × 5–10 × 3.5–6.5 μ, fusoid-ventricose and walls often flexuous, neck narrow (3.5–4.5 μ) and ending in a subacute apex; hyphae of pileus trama yellowish in KOH mostly from incrusting pigment, cuticle a layer of radial hyphae not sharply differentiated from those of flesh; clamp connections present.

Habit, habitat and distribution: On wet moss under conifers, South Fork, Lake Fork Creek, Payette Lakes, Idaho, Sept. 17, 1956, *Sm-53491*—TYPE.

The aspect of the fruiting bodies is that of *G. unicolor*, but the fibrillose annular zone and microscopic characters rule out that species. In microscopic characters it is closest to *G. cerina* var. *ampullicystis*, but differs in the rostrate cheilocystidia and aspect of the carpophores. In aspect it is very similar to *G. psathyrelloides* but that species has cheilocystidia with thicker necks and obtuse to subcapitate apices. *G. triscopa* differs in having smaller non-calyptrate spores.

16. **Galerina perangusta** sp. nov.

Pileo 5–15 mm lato et usque ad 10 mm alto, anguste conico nec repando in maturis, pallide cinnamomeo-brunneo, lamellis angustis vel moderate latis, ascendentibus, adnatis, confertis vel subdistantibus, pallide alutaceis, autoxydatione obscure ochro-brunnescentibus, demum pileo concoloribus; stipite 25–35 × 1–1.5 mm, sordide melleo atque gradatim ex integro sordide brunnescente (nec a basi apicem versus obscurato), in juvenilibus lenissime fibrilloso e velo rudimentario, veli vestigiis mox evenescentibus. Odore saporeque farinaceis; sporis 8.5–11 × 5.5–6.5 μ, levibus sed linea subtili discum suprahilarem delimitante instructis, pallide ochraceo-brunneis (KOH), callo apicali praeditis, basidiis tetrasporis; cheilocystidiis (35–)40–60 × 4–6 μ, apice saepe capitulato praeditis, infra capitulum (aut apice si capitulum abest) 2.5–3.5 μ diam., capitulo usque ad 6 μ diam.; epicute haud gelatinosa, hyphis fibulatis.

Pileus 5–15 mm broad, and up to 10 mm high, narrowly conic and remaining so, surface glabrous, moist and hygrophanous, *pale* cinnamon-brown to dull tawny, disc soon fading to cinnamon buff or more yellowish, margin striate when moist; flesh thin, concolorous with surface, taste *distinctly farinaceous*, odor similar if flesh is crushed.

Lamellae narrow to moderately broad ascending-adnate, close to subdistant, pallid buff young, becoming dark dingy ochraceous tawny when bruised, concolorous with pileus in age, edges pallid.

Stipe 2.5–3.5 cm long, 1–1.5 mm thick, equal down to a small basal enlargement, tubular, cartilaginous, dingy honey color over all and gradually *darkening over all* to dingy tawny (*not* darkening appreciably from base upward), when very young faintly fibrillose from a rudimentary veil but all traces soon gone.

Spores 8.5–11 × 5.5–6.5 μ, inequilateral in side view, ovate in face view, smooth but with a faint ragged line marking the plage, pale ochraceous tawny in KOH, dark red-brown in Melzer's sol.; apical callus distinct; basidia 4-spored; pleurocystidia none; cheilocystidia abundant, (35–)40–60 × 4–6 × 2.5–3.5 × 4–6 μ, base equal to slightly ventricose, neck equal, apex slightly enlarged to merely blunt, hyaline to faintly yellowish in KOH, thin-walled, smooth; gill trama regular, ochraceous-tawny to merely yellowish; pileus trama with a non-gelatinous poorly differentiated pellicle and a tramal body of more or less ochraceous to cinnamon-colored incrusted hyphae; clamp connections present.

Habit, habitat and distribution: Gregarious on mossy trunk of redwood, Humbolt State Park, Calif., Nov. 30, 1956, *Sm-56007*—TYPE.

The farinaceous taste, essentially smooth spores, elongate, narrow cheilocystidia mostly with enlarged apices, slight veil and changing gills are distinctive.

17. **Galerina pseudocerina** sp. nov.

Pileo 5–8 mm lato, ochraceo-brunneo; lamellis distantibus latis; stipite 20–30 × 1 mm apice pruinoso, velo inchoato, sporis 11–14(–16) × 6.5–8(–10) μ, manifeste tuberculato-asperulatis, intense brunneis (KOH), disco suprahilari levi interdum indistincto vel limitibus irregularibus instructo, basidiis 2- vel 4-sporis; cheilocystidiis ventricoso-capitatis, parvis, parte superiore capituloque angustis (3–5 μ), 18–26 × 6–9 μ, fibulis praesentibus.

Pileus 5–8 mm broad, obtusely conic becoming broadly conic, glabrous, moist, hygrophanous, striate, ochraceous tawny; flesh thin, odor none, taste mild.

Lamellae *distant, broad*, adnate, pale ochraceous tawny, edges even.

Stipe 2–3 cm long, 1 mm thick near apex, pruinose above, naked lower down at maturity, at first with a few fibrils from the rudimentary veil, pale honey-color over all.

Spores 11–14(–16) × 6.5–8(–10) μ, broadly inequilateral in side view, oval in face view, distinctly tuberculate-roughened, rich tawny in KOH, plage often difficult to see, small and its boundary irregular; basidia 20–25 × 8–19 μ, clavate, readily collapsing, 2- and 4-spored;

pleurocystidia none; cheilocystidia ventricose-capitate, 18–26 × 6–9 × c. 2.5 × 3–5 μ, neck very narrow and capitellum small, smooth, hyaline to yellowish in KOH; gill trama subregular, ochraceous in KOH; pileus trama fulvous from pigment incrusting the hyphae as well as color in wall; cuticle of narrower hyphae than those of the hypoderm but not gelatinous; clamp connections present.

Habit, habitat and distribution: Gregarious on *Dicranum*, Trout Lake, San Juan Mountains, Colorado, Aug. 14, 1956, *Sm-52182*—TYPE.

The large, ornamented, richly colored, broad spores, capitate cheilocystidia and only the most rudimentary of veils are the distinguishing characters. The field aspect of the species is that of *G. cerina*, but of course the spores are very different.

Sm-52745, Trout Lake, Colorado, Aug. 23, 1956, appears to be the same. In it the basidia are 4-spored, the spores 9–12.5 × 6–7.5 μ, but nearly all are ornamented over the plage area. The fact that a very few show some indication of a smooth plage is considered significant. Clamp connections are abundant. The cheilocystidia are 20–30 × 6–8 × 2.5–3.5 × 4–6 μ, and ventricose capitate as in *G. pistillicystis*. The spores are well-ornamented, broadly ovate to elliptic in face view, and in side view subelliptic to obscurely inequilateral, and russet to "Mars brown" in KOH.

18. **Galerina rostrata** sp. n.

Pileo 6–12 mm lato, margine in juvenilibus subincurvato, glabro, lubrico-subviscido, brunneo in centro, ochraceo-brunneolo in margine striato; lamellis subdistantibus, adnatis, latis; stipite 20–25 × c. 1 mm, deorsum subincrassato, fibrillis paucis sparsis ornato sed mox denudato, in parte superiore concolori cum lamellis, in inferiore cum disco pilei. Sporis 9–11.5 × 4.7–5.5 μ, nonnullis vix manifeste angulari-ovoideis, levibus vel sublevibus sed disco suprahilari linea delimitato; basidiis tetrasporis; cheilocystidiis numerosis, 35–50 × 6–10(–11) μ, ampullaceis (basi ventricosis, apice elongatis attenuatisque ad 3–4 μ diam., obtusis), tenuitunicatis, hyalinis; epicute pilei subgelatinosa vel haud gelatinosa, eius hyphis ab hyphis hypodermii vix differentiatis, 4–8 μ diam., fibulis praesentibus.

Pileus 6–12 mm broad, plano-umbonate young and with a slightly incurved margin, becoming campanulate or remaining plano-umbonate, surface glabrous, subviscid, "tawny" on disc and "ochraceous tawny" over striate margin; flesh very thin and soft, odor and taste mild.

Lamellae subdistant, broadly adnate, broad, dingy pale ochraceous tawny.

Stipe 2–2.5 cm long, c. 1 mm thick at apex, slightly enlarged downward, with a few scattered fibrils when young, soon naked throughout, concolorous with gills above, base darker and concolorous with disc of pileus.

Spores 9–11.5 × 4.7–5.5 μ, more or less inequilateral in side view, in face view ovate, some obscurely angular-ovate, pale ochraceous tawny in H_2O fresh, tawny in KOH, dark red-brown in Melzer's, smooth or practicaly so (under oil) but plage determined by a faint line; basidia 4-spored; pleurocystidia none or only near gill edge and similar to cheilocystidia; cheilocystidia abundant, 35–50 × 6–10(–11) μ, slightly ventricose at the base or over lower part, tapered to a long narrow (3–4 μ in diam.) neck which ends in an obtuse or rarely slightly enlarged apex, walls thin, smooth, hyaline in KOH; gill trama of subparallel hyphae, walls of hyphae ochraceous to pale cinnamon in KOH (good sections showing almost hyaline walls), incrustations absent to inconspicuous; pileus trama with a pellicle of slightly gelatinous hyphae (or entirely non-gelatinous) and 4–8 μ in diam., walls incrusted with pigment (no true differentiation of the pellicle), flesh beneath of larger hyphae more highly colored in KOH; clamp connections present.

Habit, habitat and distribution: Gregarious on a mossy log, Prairie Creek State Park, Orick, Calif., Dec. 5, 1956, *Sm-56364*—TYPE.

This species belongs in stirps *Mycenopsis* where it falls in the vicinity of *G. vexans, G. lubrica,* etc. It is distinguished by its cheilocystidia.

19. **Galerina subcerina** sp. n.

Pileo 3–10 mm lato, obtuse conico vel companulato, raro convexo, ochraceo-brunneo; lamellis latis, distantibus; stipite omnino pallide melleo, 10–30 × 1.5 mm; velo nullo; sporis 9–11.5 × 5.5–7 μ, calyptratis; cheilocystidiis eis *G. cerinae* v. *cerinae* simillimis.

Pileus 3–10 mm broad, obtusely conic to campanulate, rarely convex, margin straight, surface glabrous, moist, and hygrophanous, ochraceous tawny and striate, fading to pale buff; odor and taste not recorded.

Lamellae broad, distant, adnate, nearly concolorous with the moist pileus when mature.

Stipe 1–3 cm long, filiform to 1.5 mm thick, equal, pale honey color overall, pruinose at apex, naked over the remainder, no veil present.

Spores 9–11.5 × 5.5–7 μ, ovate in face view, somewhat inequilateral in side view, calyptrate, tawny in KOH, darker red-brown in Melzer's, callus very inconspicuous; basidia 4-spored; pleurocystidia absent or present only near the gill edge, similar to cheilocystidia; cheilocystidia abundant, 26–38(–50) × 7–12 × 4–6 × 5–9 μ, base equal to slightly ventricose, apex obtuse to capitate, smooth, hyaline to pale yellowish in KOH; gill trama fulvous in sections revived in KOH; pileus trama fulvous in KOH (individual hyphae with ochraceous incrusting pigment), pellicle of appressed hyphae 5–8 μ in diam. and *slightly* gelatinous in KOH as well as ochraceous in color, clamps present.

Habit, habitat and distribution: Usually only a few fruiting bodies in a small area; on mossy conifer logs, Aroostook County, Maine, summer and fall; near Guerette, Aug. 25, 1956, *Bigelow 4379*—TYPE.

The distinctly smaller spores and more capitate cheilocystidia separate this from *G. evelata*.

20. **Galerina subtruncata** sp. n.

Pileo 10–25 mm lato, brunneo vel ochraceo-brunneo; lamellis latis, subdistantibus; stipite flavido, deorsum obscure rubido-brunneo, tenuiter fibrilloso e velo pallido; sporis 7–9.5 × 4.5–5 μ, levibus, poro germinativo instructis subtruncatisque; basidiis tetrasporis; cheilocystidiis 23–34 × 7–9 μ, anguste ventricosis, parte superiore subattenuata; epicute haud gelatinosa; fibulis praesentibus.

Pileus 10–25 mm broad, convex, expanding to broadly convex, surface glabrous, moist and hygrophanous, tawny to ochraceous tawny when moist, fading to pinkish buff and appearing slightly fibrillose, striate moist; flesh thin, odor and taste not recorded.

Lamellae broad, subdistant, broadly adnate to short decurrent, ochraceous tawny, edges even.

Stipe 2–3 cm long, 2–2.5 mm thick, equal, fragile, yellowish above, dark reddish brown below, thinly fibrillose up to near apex from remains of a thin pallid veil.

Spores 7–9.5 × 4.5–5 μ, obscurely inequilateral to subovate in side view, ovate in face view, thick-walled, smooth, tawny to ochraceous tawny in KOH, *with a distinct apical pore causing apex to appear obscurely truncate;* basidia 4-spored, 20–23 × 7–8 μ, hyaline in KOH; pleurocystidia absent; cheilocystidia 23–34 × 7–9 μ, narrowly ventricose with a slightly narrowed neck and obtuse apex, hyaline, thin-walled, smooth; gill trama somewhat interwoven, yellowish to hyaline in KOH; pileus trama hyaline and homogeneous beneath a *surface layer of ochraceous-tawny incrusted hyphae,* with single hyphae or fascicles appressed to surface and the cells cylindric to somewhat inflated; clamp connections present.

Habit, habitat and distribution: Scattered on wet soil among mosses in a wet area, St. Andrew's Creek, Mt. Rainier National Park, Sept. 24, 1952, *Sm-40199*—TYPE.

This species belongs in *Porospora* of *Galerina* where because of its small spores it comes close to *G. lateritia*.

21. GALERINA STYLIFERA var. **badia** var. nov.

A varietate typica differt coloribus: Pileo badio, demum ochraceo-brunneo; stipite ferrugineo-brunneo basin versus melleoque ad apicem, in exsiccatis unicolori brunneo.

Pileus 1–2 cm broad, convex, the margin incurved, expanding to broadly convex or nearly plane, glabrous, viscid shinning, dark red-brown (badious) but gradually becoming paler (to ochraceous-tawny) and finally fading to cinnamon-buff, striate when moist.

Lamellae subdistant, broad, adnate, yellowish young, becoming ochraceous-tawny.

Stipe 2–3.5 cm long, 2.5–3 mm thick, equal, *rusty brown* below, honey-color above, apex pruinose, glabrous except for a thin fibrillose zone from the broken veil, naked and watery in appearance at maturity; *when dried evenly tawny over all* or base only slightly darker.

Spores 6–7 × 3.8–4.3 μ, ellipsoid, smooth, pale tawny in KOH and not darkening in Melzer's sol.; basidia 4-spored; pleurocystidia none; cheilocystidia 18–26 × 3–7 × 2–3 × 5 μ, capitate, hyaline, smooth; gill trama with a floccose central cinnamon-colored (in KOH) strand of tissue flanked by a gelatinous subhymenial zone on either side; pileus trama with a distinct gelatinous pellicle of narrow hyaline hyphae, remainder floccose and pale cinnamon to ochraceous; clamp connections present.

Habit, habitat and distribution: Gregarious on conifer debris, Granite Creek, Kaniksu Nat. Forest, Idaho, Oct. 6, 1956, *Sm-54112*—TYPE.

The red-brown pilei and difference in the color change of the stipe distinguish this variety from the type variety.

22. GALERINA STYLIFERA var. **velosa** var. nov.

A *G. stylifera* var. *stylifera* differt veli abundantia.

Pileus 2.5–3.5(–5) cm broad, convex with an inrolled margin, expanding to broadly convex or nearly plane, surface glabrous or margin at first decorated with veil remnants, viscid, pale "ochraceous-tawny" to "buckthorn-brown," moist, fading to cinnamon-buff and slightly darker when dried; flesh moderately thick and pliant, odor and taste not distinctive.

Lamellae "buckthorn-brown" becoming nearly "ochraceous-tawny," more or less concolorous with cap, moderately close, 2–3 tiers of lamellulae, broad (up to 6–7 mm), depressed-adnate, seceding, edges even.

Stipe 4–6 cm long, 3–6 mm thick at apex, evenly enlarged downward, rather firm, solid to tubular, pallid and silky above the rather pronounced fibrillose annular zone, coated with patches or zones of fibrils below, brownish toward the base but not discolored markedly in drying.

Spores 6–7 × 4 μ, essentially ellipsoid, smooth, apical pore not evident, basidia 4-spored, 20–22 × 6 μ, hyaline in KOH; pleurocystidia none; cheilocystidia abundant, 20–26 × 4–7 × 2–3.5 × 3–4 μ, narrowly ventricose below, hyaline, thin-walled; gill trama with a broad subparallel ochraceous-tawny floccose zone flanked by very narrow gelat-

inous subhymenial layers or these not showing at all; pileus trama of floccose-interwoven hyphae pale tawny near the gills and hyaline near the cuticle but a narrow tawny hypodermium present between the hyaline area and the gelatinous hyphae; pellicle thick, of narrow gelatinous hyaline to yellowish hyphae; clamp connections present.

Habit, habitat and distribution: Scattered to cespitose on decayed conifer wood, Idaho, 1954, *Sm-47201*—TYPE.

This is a large form consistently mistaken in the field for *G. autumnalis*.

23. GALERINA VITTAEFORMIS var. **pachyspora** var. nov.

A varietate typica differt sporis latioribus; basidiis (1–)2(–3)-sporis tantum.

Pileus c. 12 mm broad, conic with rounded apex, with striate margin, light brown to brown.

Lamellae cream colored to pale brown, adnate, slightly sinuate (?).

Stipe yellow brown, darker at base, cartilaginous, fistulose, without veil, up to 20 mm long, 1–2 mm thick.

Spores 11.7 × 7.5–8.2 μ, slightly compressed, typically about 7.5 μ in side view and about 8.2 μ in face view, almond-shaped with asymmetric outline in profile, when mature well colored and with heavy verrucose exosporial ornamentation, many pale immature spores present on hymenium, apex with a plage and sometimes extended to a slight beak, with a double wall which is sometimes very thick; basidia 30–34 × 8–8.5 μ, mostly 2-spored but a few 1- or 3-spored; pleurocystidia scattered, hyaline, ampullaceous with ventricose base and usually long-cylindric apex, 48–59 × 8.8–11.8 μ, lower portion of the thinned apex 6.2–8.2 μ in diam., upper part 4–6.3 μ; cheilocystidia numerous, similar to pleurocystidia; hymenophoral trama of rather broad parallel hyphae; pellicle of pileus of thin (1.5 μ) smooth hyphae which are rather loosely arranged but not visibly gelatinized forming a thin but distinct layer; hypodermium somewhat pigmented, of broader and more compactly arranged parallel or subparallel hyphae; pileocystidia absent; caulocystidia present and similar to the pleurocystidia; clamp connections regularly present.

On a moss bed consisting of various species but no *Sphagnum* noted, though possibly the whole bed extending over a layer of peat. West Point, Macquarie Is., March 31, 1953, *Bunt*, TYPE (LIL, MICH), also in a *Juncus* swamp at Half Moon Bay, Macquarie Is., Jan. 31, 1951, *Bunt* (LIL).

The broad spores remind one of *G. atkinsoniana*, but the lack of pileocystidia excludes it from that species.

24. GALERINA TRISCOPA f. **longicystis** f. nov.

A forma typica differt cystidiis maturis elongatioribus.

Pileus 6–12 mm broad, sharply conic with an incurved margin, becoming expanded but retaining a prominent conic umbo, evenly cinnamon-brown to russet (very dark rusty brown), odor and taste not recorded.

Lamellae subdistant, broadly adnate, broad, *dark rusty brown*, edges denticulate.

Stipe 2 cm long, 1.5 mm thick, equal, badious below, paler above (dark wine-red as dried), surface of lower part with a few grayish fibrils from a thin veil, soon naked, apex slightly pruinose.

Spores 6–7.5 × 4–4.5 μ, inequilateral in side view, ovate in face view, warty roughened (under oil), with a smooth plage, dark dull ochraceous tawny in KOH; basidia 4-spored; pleurocystidia none or near edge and similar to cheilocystidia, cheilocystidia 30–40 × 9–12 μ, and fusoid-ventricose, elongating to 50–60 μ and then subcylindric with flexuous walls, seldom enlarged at apex; basal part *in age* pale ochraceous tawny and the elongated cells agglutinated into fascicles finally; pileus trama lacking a distinct pellicle, all hyphae heavily incrusted, clamp connections present.

Habit, habitat and distribution: Scattered on a very decayed mossy conifer log, Payette Lake, Idaho, Aug. 30, 1954, *H. Bigelow* (Sm-47110)—TYPE.

The elongating cheilocystidia at once distinguish this fungus from *G. triscopa* f. *triscopa* even if one disregards the color. The two are identical in stature.

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THE BIOSYNTHESIS OF CHLORAMPHENICOL. III. EFFECTS OF MICRONUTRIENTS ON SYNTHESIS

VIRGINIA GALLICCHIO AND DAVID GOTTLIEB

Although many studies of the mineral requirements of fungi have been reported, there are few published investigations on the needs of actinomycetes for added mineral salts in synthetic media. Most of the work on the micronutrient requirements of this group has dealt with production of vitamin B_{12} and streptomycin, grisein or candicidin by *Streptomyces griseus* (Chesters and Rolinson, **1951**; Thornberry, **1948**; Thornberry and Anderson, **1948**; Reynolds and Wakeman, **1948**; Acker and Lechevalier, **1954**). Iron and zinc seem to be quite generally required for the optimum growth of this organism, and for the best production of antibiotics; in some cases copper or manganese increased the zinc-iron stimulation.

In the course of preparing cell-free extracts of *S. venezuelae* for enzyme studies it was noted that the synthetic medium used (Gottlieb *et al.*, **1954**), which had been heated and filtered prior to sterilization, did not support the synthesis of chloramphenicol. It seemed likely that precipitation of magnesium phosphate during heating of the medium had also removed certain cations essential to chloramphenicol synthesis but not to growth of the organism.

MATERIALS AND METHODS

The control medium for all experiments was a glycerol-lactate medium (Gottlieb *et al.*, **1954**), in which a mixture of 8 cations (Zn, Fe, Mn, Cu, B, Mo, Co, and Ga, each 2.6×10^{-5} M) was substituted for the minor element solution previously used. The experimental basal medium was the glycerol-lactate medium prepared without the addition of minerals and treated prior to dispensing and sterilizing by the $CaCO_3$ method (Steinberg, **1919**) or the Al_2O_3 method (Donald *et al.*, **1952**). All glassware was Pyrex and had been cleaned with chromic acid and well rinsed with Pyrex-distilled water before use. Pyrex-distilled water was also used for preparation of the basal medium and salt solutions, and for washing the mycelial inoculum. The mineral salts were prepared as molar or 10^{-1} M solutions in 10^{-1} N HCl and were diluted and

added to 75 ml treated basal medium to give the desired final concentration. Each experimental medium was evenly divided among triplicate 125 ml Erlenmeyer flasks and the flasks were plugged and sterilized at 15 lb steam pressure for 15 min.

The inoculum medium was the glycerol-lactate medium containing a microelements solution (Thornberry and Anderson, **1948**). The medium was heated and filtered prior to being dispensed; this treatment apparently removed all but the minimum amount of minerals that would allow good growth without chloramphenicol production and minimized storage of excess minerals in the mycelium. Spores from two 3- to 5-day-old chloramphenicol-producing cultures of *S. venezuelae* strain R-1

TABLE I

EFFECT ON GROWTH AND CHLORAMPHENICOL PRODUCTION OF THE ELIMINATION OF SINGLE MINERALS FROM THE $CaCO_3$-TREATED MEDIUM

MINERALS	EFFECT ON GROWTH [a]	EFFECT ON PRODUCTION [a]
each 2.6×10^{-5} M	%	%
none	40	<16[b]
Zn, Fe, Mn, Cu, B, Mo, Co, Ga	81	66
same, minus Zn	74	<16[b]
same, minus Fe	71	<16[b]
same, minus Mn	105	70
same, minus Cu	73	143
same, minus B	93	80
same, minus Mo	74	90
same, minus Co	82	87
same, minus Ga	63	87

[a] Expressed as percent of that found in control flasks containing untreated medium plus all 8 minerals. Production of chloramphenicol in these flasks averaged 30 μg/ml and the mycelium weight averaged 2.83 mg/ml.

[b] Minimum detectable chloramphenicol concentration was 5 μg/ml; these cultures had no detectable antibiotic.

on tryptone-glycerine-agar slants (Oyaas *et al.*, **1950**) were suspended in sterile water and added to a 500 ml flask containing 100 ml of medium. After 48 hr on a reciprocal shaker at 26° C, the mycelium was removed by centrifugation, washed twice with sterile water and resuspended in sterile water. Each culture flask was inoculated with 0.5 ml of this mycelial suspension and incubated on the shaker for 4 to 5 days.

Grown cultures were filtered through washed, Whatman no. 1, 9 cm papers; culture filtrates were collected in tubes and assayed for chloramphenicol by the paper disc method (a modification of the method of Smith *et al.*, **1950**). All mycelia adhering to the walls of the culture

flasks were washed onto the filter paper. The papers were dried at 80° and weighed; similarly washed papers served as tares.

RESULTS

The control medium supported no production of chloramphenicol in the absence of added minerals, although the weight of mycelium formed was 83 per cent of that in control flasks containing the 8 micronutrients studied. Growth in the $CaCO_3$-treated medium, in the absence of added micronutrients, was only 40 per cent of that in the controls (TABLE I). Good growth and fair chloramphenicol production were obtained when all 8 minerals were added to the treated medium, but the elimination of either Zn or Fe from that addition resulted in the absence of antibiotic production.

TABLE II

EFFECT ON GROWTH AND CHLORAMPHENICOL PRODUCTION OF MINERALS ADDED SINGLY AND IN PAIRS TO THE $CaCO_3$-TREATED MEDIUM

MINERAL(S)	EFFECT ON GROWTH [a]	EFFECT ON PRODUCTION [a]
each at 10^{-4} M	%	%
none	42	<16[b]
Zn, Fe, Mn, No, Cu, Co, B, Ga	86	83
Zn only	34	<16[b]
Fe only	67	<16[b]
Each of the other minerals alone	38–65	<16[b]
Zn + Fe	83	100
Each other paired combination	34–81	<16[b]

[a] Expressed as percent of that found in control flasks containing untreated medium plus all 8 minerals. Production of chloramphenicol in these flasks averaged 30 μg/ml and the mycelium weight averaged 2.89 mg/ml.

[b] Minimum detectable chloramphenicol concentration was 5 μg/ml; these cultures had no detectable antibiotic.

When each of the minerals was added singly to the treated medium, growth was poor to fair, but no addition could support chloramphenicol production (TABLE II). Chloramphenicol synthesis was obtained only with the addition of Zn and Fe, of all possible pairs of the 8 minerals, and this production equaled that in the control flasks. The addition of 1, 2, or 3 other minerals to the Zn-Fe combination decreased growth somewhat and did nothing to improve antibiotic production.

Varying the concentrations of Zn and Fe from 10^{-2} to 10^{-6} M, simultaneously or one at a time, showed that the combination of 10^{-3} M Fe and 10^{-4} M Zn was optimum for production. With these concentrations of Fe and Zn, Mg concentrations from 5×10^{-6} to 10^{-2} M increased proportionately the total growth of the organism. However,

chloramphenicol production, measured either as μg/ml or as μg/mg mycelium, showed the optimum Mg concentration to be 10^{-4} M (TABLE III). In the absence of added Mg, 23 per cent of normal production was obtained with the substitution of Mn at 10^{-2} M; lower concentrations of Mn allowed no detectable synthesis, although growth in these cases was good. The combination of Mn and Mg was no better for growth or production than Mg alone.

Co-precipitation with alumina was compared with calcium carbonate as a method for removing essential trace elements from the synthetic

TABLE III

EFFECT OF MAGNESIUM CONCENTRATION IN THE $CaCO_3$-TREATED MEDIUM ON GROWTH AND PRODUCTION OF CHLORAMPHENICOL

MINERALS	MOLAR CONCENTRATION	EFFECT ON GROWTH [a]	EFFECT ON PRODUCTION [a]
		%	%
Medium with Mg:			
none	0.008	23	<16[b]
Zn, Fe, Mn, Cu, B, Mo, Co, Ga (each at 10^{-4} M)	0.008	75	95
Medium without Mg:			
Zn, 10^{-4} M			
Fe, 10^{-3} M no Mg	—	35	13
Fe, 10^{-3} M + Mg	0.00005	70	137
Fe, 10^{-3} M + Mg	0.0001	82	173
Fe, 10^{-3} M + Mg	0.0005	114	90
Fe, 10^{-3} M + Mg	0.001	124	70
Fe, 10^{-3} M + Mg	0.0025	116	57
Fe, 10^{-3} M + Mg	0.005	135	73
Fe, 10^{-3} M + Mg	0.0075	155	77
Fe, 10^{-3} M + Mg	0.01	152	60

[a] Expressed as percent of that found in control flasks containing untreated medium plus all 8 minerals. Production of chloramphenicol in these flasks average 19 μg/ml and the mycelium weight averaged 2.74 mg/ml.

[b] Minimum detectable chloramphenicol concentration was 5 μg/ml; these cultures had no detectable antibiotic.

medium (TABLE IV). After alumina treatment, growth in the absence of added micronutrients was considerably better than after calcium carbonate, and chloramphenicol production with added minerals was then equal to that in the controls.

There had been numerous indications in the earlier studies with carbonate-treated media that Cu was toxic, possibly to growth but certainly to production. The effect of Cu at concentrations of 10^{-4} to 10^{-7} M was studied in the medium treated with alumina; there was no deleterious effect on growth or production at levels of 5×10^{-5} M or lower, but 10^{-4} M Cu decreased chloramphenicol production 35 percent.

Some evidence was obtained in a brief experiment not described above that the function of lactate in the synthetic medium is partially to form a protective complex with Zn during autoclaving. Production of chloramphenicol, when sterile lactate was added to a lactate-free medium post-sterilization, was 25 percent lower than in controls; when the $ZnSO_4$ solution was autoclaved separately there was no such difference. Although lactate is essential for chloramphenicol production in the glycerol synthetic medium, little of it is removed from the medium during growth; studies with uniformly labeled lactate-C^{14} show that the

TABLE IV

EFFECT OF TWO METHODS FOR REMOVAL OF MICRONUTRIENTS FROM MEDIUM ON GROWTH AND CHLORAMPHENICOL PRODUCTION

MEDIUM	EFFECT ON GROWTH [a]	EFFECT ON PRODUCTION [a]
	%	%
Al_2O_3-treated:		
+ no minerals	72	<16[b]
+ 8 minerals, each 10^{-4} M	100	106
+ 7 minerals, each 10^{-4} M (no Cu)	102	100
$CaCO_3$-treated:		
+ no minerals	52	<16[b]
+ 8 minerals, each 10^{-4} M	98	80
+ 7 minerals, each 10^{-4} (no Cu)	92	69

[a] Expressed as percent of that found in control flasks containing untreated medium plus all 8 minerals. Production of chloramphenicol in these flasks average 35 μg/ml and the mycelium weight averaged 2.53 mg/ml.

[b] Minimum detectable chloramphenicol concentration was 5 μg/ml; these cultures had no detectable antibiotic.

labeling of the chloramphenicol subsequently isolated is very weak and, probably, is evenly distributed.

DISCUSSION

Biochemists and biologists are becoming increasingly aware of the many and varied roles played by metallic ions as essential components of enzyme systems. The roles of Fe and Cu as the functional centers of certain oxidases are well known; so is the presence of Co in the coenzyme formed from vitamin B_{12}. Mg and Mn are necessary as activators of many enzymes involved in phosphate transfer, dehydrogenation or decarboxylation; Zn can replace Mg in activating some of these systems.

No such cation forms an integral part of the chloramphenicol molecule; however, some metallic ions must necessarily be involved in the enzyme systems utilized at some stage in the biosynthesis of the antibiotic, since Zn and Fe at least have been shown to be essential for antibiotic production. In these studies no attempt was made to determine the residual concentrations of the various micronutrients in the treated media; although levels of the micronutrients still present were adequate for growth, there was no chloramphenicol production detectable by either chemical or biological means in the absence of added minerals. It would seem, therefore, that chloramphenicol biosynthesis requires enzyme systems not directly involved in the growth of the organism, and that these systems are dependent upon rather high concentrations of Fe and Zn. It seems likely, also, that the Mg-requiring enzymes necessary for biosynthesis are different from those necessary for growth alone, since Mn can readily substitute for Mg only as far as growth is concerned.

Practical application of these data can be made, in a change of the formulation of the synthetic medium used in our studies on chloramphenicol biosynthesis. The concentration of Mg should be decreased from 8×10^{-3} M now used to about 10^{-4} M when optimum chloramphenicol production rather than optimum growth is desired; the concentration currently used is satisfactory for growth alone. The concentrations of Fe and Zn should be increased from 4×10^{-5} M each to 10^{-3} M and 10^{-4} M respectively. The Cu and Mn of the minor-element mixture now in use can be eliminated, since quantities of these minerals sufficient for good growth and production must occur as contaminants of the other salts in the medium.

SUMMARY

The effects of the microelements Zn, Fe, Mn, Mo, Co, Cu, B, and Ga and the macroelement, Mg, on growth and chloramphenicol production by *Streptomyces venezuelae* were studied. Elimination of Zn or Fe from the microelement mixture added to a $CaCO_3$-treated synthetic medium resulted in the absence of chloramphenicol production. No one mineral in the synthetic medium would support antibiotic production, but the addition of Zn with Fe resulted in good production. Mg at levels of $5 \times$ and 10×10^{-5} M was best for chloramphenicol production; levels of $7.5 \times$ and 10×10^{-2} M were optimum for growth. Mn could replace Mg in the growth requirement, but only an Mn concentration of 10^{-2} M allowed any chloramphenicol production. Presence or absence of the other microelements studied, singly or in groups, had little effect on

growth or production in the medium containing optimum concentrations of Zn, Fe, and Mg.

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A NEW CALCARISPORIUM PARASITIC ON OTHER FUNGI [1]

H. L. Barnett

(WITH 10 FIGURES)

During the routine culturing of oak twigs in 1954, testing for the presence of oak wilt, a pure white imperfect fungus was found growing over the dark mycelium of *Dothiorella quercina* (Cke. and Ell.) Sacc., on which it appeared to be parasitic. Attempts to grow this fungus in pure culture on several different agar media failed. The spores germinated readily on media containing natural products and usually produced secondary spores, but normal development of the mycelium and conidiophores occurred only in the presence of a host fungus. It was established that a balanced type of parasitism exists between the parasite and its host. The host appears to be harmed but little in this relationship.

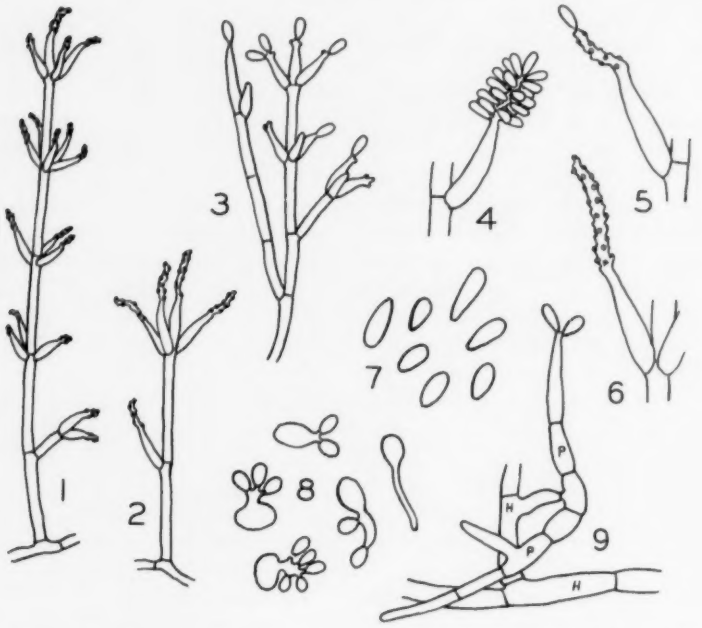
Five additional isolates of this parasite were obtained during 1956, all from *Dothiorella* sp. from different oak trees. Characteristic of this fungus are the pure white mycelium, conidiophores and conidia, the simple or branched conidiophores bearing sporogenous cells (phialides) singly, in pairs, or in whorls of 3 to 6, and the production of a cluster of conidia on the extended, fertile, apical portion of the sporogenous cells. The conidia are produced successively on new growing tips and are borne on short, blunt wart-like or peg-like projections.

A search through the available literature has failed to reveal any species to which this fungus could be assigned. In the development of the conidiophores, the frequent verticillate arrangement of the sporogenous cells and the narrowly obclavate sterile portion of the sporogenous cells, it is similar to *Calcarisporium arbuscula* Preuss, the only known species of this genus (Hughes, 1951).[2] However, it differs from this species in the larger conidiophores and the prolonged extension of the fertile portion of the sporogenous cells and in the blunt wart-like or peg-like projections bearing the spores.

[1] Published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 572.

[2] Hughes, S. J. 1951. Studies on micro-fungi. IX. Calcarisporium, Verticicladium, and Hansfordia (gen. nov.). Comm. Myc. Inst. Myc. Papers No. 43, pp. 1–25.

Dr. S. J. Hughes, who made a brief examination of living material of this species, agreed that its taxonomic position was near *Calcarisporium*, but suggested (in personal communication) the genus *Hansfordia* Hughes as a possible disposition of this fungus. In discussing the differences between *Hansfordia* and *Calcarisporium* Hughes (**1951**) states:



FIGS. 1–9. *Calcarisporium parasiticum*. 1. An unusually well developed conidiophore from old culture, showing verticillate arrangement of sporogenous cells, × 200. 2. Short conidiophore with whorl of sporogenous cells only at apex, × 250. 3. Young branched conidiophore showing development of sporogenous cells and first spores, × 350. 4. Single sporogenous cell and cluster of conidia, × 800. 5, 6. Sporogenous cells showing spore scars after spores have been removed, × 1,000. 7. Conidia, × 1,000. 8. Germinating conidia with germ tubes and secondary conidia, × 500. 9. Small portion of host mycelium (H) and method of contact with parasite (P), which is beginning to grow and to produce its first conidia, × 600.

"The main character which seems to differentiate the species classed here in the genus *Hansfordia* from other morphologically related genera is the denticulate nature of the more or less cylindrical sporogenous cells. These denticles are not restricted to the immediate, sometimes swollen apex, as in the subulate sporogenous cells of *Calcarisporium arbuscula;*

in any case the latter usually has simple conidiophores with regular verticils of sporogenous cells inserted directly on the main stipe." He further points out that true verticils, i.e., three or more laterals arising from the same level of the conidiophore, are absent in the genus *Hansfordia*.

It would appear to this author that the obclavate sporogenous cells with the slender, fertile, apical region and the common verticillate arrangement of the sporogenous cells on the main axis or branches of the larger conidiophore would exclude this species from the genus *Hansfordia*. It seems that the logical disposition of this fungus is in the genus *Calcarisporium*, at least until more is known about closely related

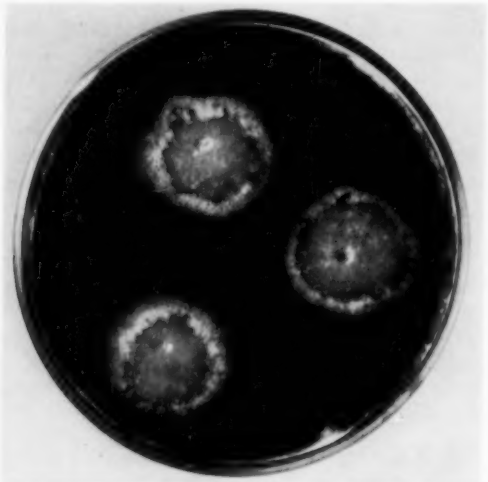


FIG. 10. *Calcarisporium parasiticum* (white mycelium) growing on and spreading over the dark mycelium of *Physalospora obtusa*.

genera and species. There is a striking resemblance between this species and the illustrations of *C. arbuscula* (Hughes, **1951**, *fig. 1*). It is, therefore, concluded that this fungus represents an undescribed species closely related to *C. arbuscula*.

The purpose of this paper is to describe and illustrate this species under the name *Calcarisporium parasiticum* n. sp. The species epithet is chosen to describe its strict parasitic habit on certain other species of fungi. The characteristics of this species are illustrated in FIGS. 1–10. A study of the host range, the host-parasite relationship and other factors affecting the growth of the parasite is in progress and the results will be reported in a later publication.

Calcarisporium parasiticum n. sp.[3]

Mycelium hyalinum, septatum, non diffusum, 1.5–3 μ latum; conidiophori hyalini, simplices vel ramosi, axe principali 3–4.5 μ lato, glabro vel minute verrucoso; cellulae sporogenae (phialides) singulae, binae vel 3–6-verticillatae, anguste obclavatae, parte basali sterili, apicali fertili, indefinite proferenti, racemum laxum conidiorum gerenti; hili in clavulis brevibus verruciformibus prominentes; conidia apicaliter ordinatim e locis auctus novis nata, hyalina, sicca, anguste obovata vel elliptica, plerumque ad basim angustioria, 2.5–4 × 6–10 μ; mycelium fungorum aliorum quorundam incolens et parasitans.

Mycelium hyaline, septate, not extensive, sparsely branched, mostly 1.5–3 μ wide, growing over the mycelium of host fungi and making contact with the hyphae by means of short lateral branches, but not penetrating the host cells; conidiophores hyaline, variable, short and simple, or long and irregularly branched, reaching a height of 0.5 mm or more; the main axis 3–4.5 μ wide, smooth or roughened with minute warts, thin to thick walled; sporogenous cells (phialides) arising singly from the mycelium, or from the conidiophore, or in pairs, or in whorls of 3 to 6 and from different levels of the conidiophore, broader at the basal sterile portion, about 6 μ at its widest portion, narrowed toward the fertile apical portion which continues to elongate indefinitely to a length of 40 μ or more in old cultures, bearing up to 60 or more conidia in a loose ovoid or cylindrical cluster; conidia acropleurogenous, produced apically on successive growing points, hyaline, dry, not easily wet, narrowly obovoid to ellipsoid, usually narrower at the base, 2.5–4 × 6–10 μ; spore scars on short, wart-like or peg-like projections; spore germination by one or two short germ tubes usually bearing secondary conidia; normal development of mycelium occurring only in the presence of a host fungus.

Parasitic in culture on mycelium of certain species of *Physalospora, Botryosphaeria* (*Sphaeropsis* and *Dothiorella*) and *Coniothyrium*. Known only from six isolates found on cultures of *Dothiorella quercina* from oak trees in West Virginia. TYPE culture found Nov. 26, 1956, growing on a culture of *Dothiorella quercina* Cke. and Ellis isolated from sample of black oak collected in Morgan County, West Virginia, Nov. 13, 1956; West Virginia University fungus culture collection no. 1198. Cultures have been deposited in the American Type Culture Collection, the Centraalbureau voor Schimmelcultures and the West Virginia Mycological Culture Collection.

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[3] The Latin translation was kindly furnished by Miss Edith Cash.

LIFE HISTORY STUDIES OF MYCOSPHAERELLA TASSIANA AND M. TYPHAE[1]

MARGARET E. BARR[2]

(WITH 20 FIGURES)

Two species of *Mycosphaerella*, *M. tassiana* (de Notaris) Johanson and *M. typhae* (Lasch) Lindau, were grown in agar culture and produced mature fruiting bodies. The strain of *M. tassiana* was isolated as a single-spore culture from an ascostroma on a species of *Pedicularis* (659a) collected in Garibaldi Park, British Columbia. The strain of *M. typhae* was isolated also as a single-spore culture, from an ascostroma on leaves of *Typha* sp. (1536a) collected in Michigan.

CULTURAL STUDIES

Both fungi were isolated and grown on Leonian's agar. *M. typhae* grew well and matured on this medium as well as on some small portions of sterilized leaves of *Typha* which were placed on the agar surface at the edge of the fungus colony. Mycelial penetration and ascostromatal development were readily observed. However, a 0.2% maltose-yeast-extract agar was found to be more suitable for the complete maturation of *M. tassiana*. Ascostromata failed to develop on basal media supplemented with malt extract, ribose nucleic acid, and casamino digest.

Low temperature has been shown to be a factor in the formation of ascocarps in several genera. Vaughan (**1916**) found this to be so for *Mycosphaerella pinodes*, as did Burt (**1936**) and Jacques (**1941**) for species of *Didymellina* (now treated as a subgenus of *Mycosphaerella* (von Arx, **1949**)). In *Pleospora*, Simmons (**1952**) and Wehmeyer (**1954**) both presented evidence to show that a period of low temperature is necessary for ascostroma formation. Sproston and Pease (**1957**) recently reported on the influence of thermoperiods on the sexual fruit-

[1] Papers from the Department of Botany, University of Michigan. Based on a portion of a dissertation submitted to the Graduate School of the University of Michigan in partial fulfillment of the requirements for the Ph.D. degree.

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ing stage of *Sclerotinia trifoliorum*. In the present study of *M. tassiana*, I found that a period at low temperature was necessary to induce fruiting. On the other hand, no cold period was necessary for the production of ascostromata in *M. typhae*, and in fact, development was retarded at low temperatures.

TABLE 1 indicates the rate and sequence of development in *M. tassiana*, as determined by examination of water mounts, squash mounts stained in propriono-aceto-carmine, and prepared sections. A distinct pattern is noted in the early development of the colonies. The first

TABLE 1

CORRELATION OF STAGES IN DEVELOPMENT OF MYCOSPHAERELLA TASSIANA WITH VARIABLE LENGTH OF TIME AT 5° C

Total age in weeks	Weeks at 5° before removal to room temperature							
	0	2	3	4	5	6	10	17
1	V	V	V	V	V	V	V	V
2	V	S	S	S	S	S	S	S
3	V	S	S	S	S	S	S	S
4	D	N	N	N	N	N	N	N
5	—	D	N	N	N	N	N	N
6	—	—	D	N	N	N	N	N
7	—	—	—	D	N	N	N	N
8	—	—	—	—	D	F	N	N
9	—	—	—	—	—	M	N	F
10	—	—	—	—	—	SP	F	F
11	—	—	—	—	—	—	F	F
12	—	—	—	—	—	—	M	F
13	—	—	—	—	—	—	SP	F
14	—	—	—	—	—	—	—	F
15	—	—	—	—	—	—	—	F
16	—	—	—	—	—	—	—	F
17	—	—	—	—	—	—	—	SP

Explanation of symbols: V = vegetative growth; S = formation of ascostromata; D = disintegration of interior cells of stroma; N = multinucleate cells; F = fusion nucleus in young ascus; M = meiosis; SP = mature spores.

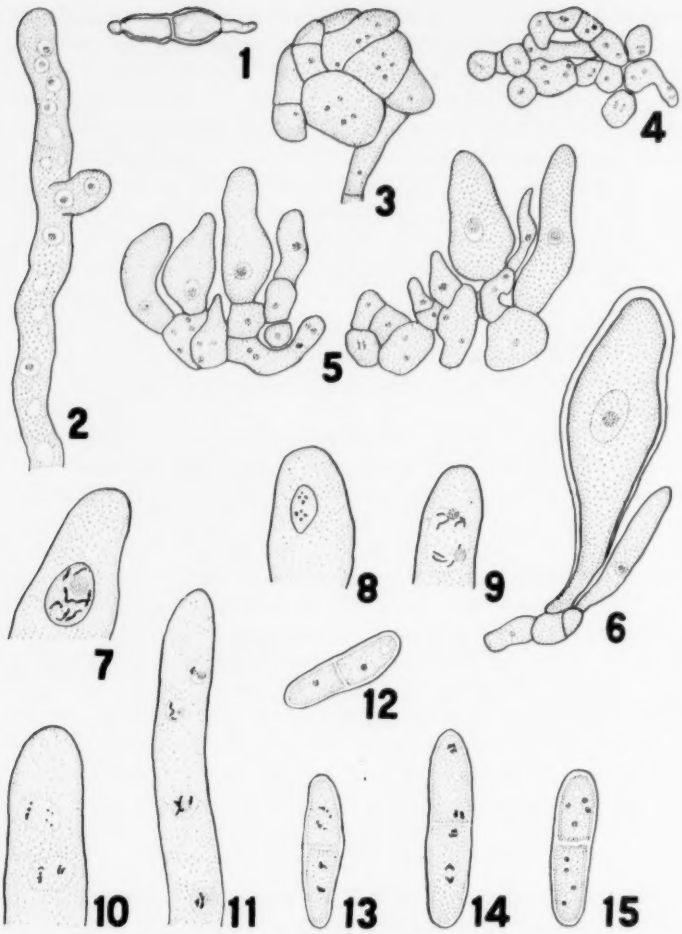
week at room temperature is necessary for the colonies to become established. Freshly inoculated plates placed immediately in the cold room do not grow. However, as soon as growth has become established the plates may be removed to the cold room. When retained at room temperature vegetative growth and conidial formation will continue for some time, whereas at 5° C these processes are greatly retarded. Within four weeks at low temperature ascostromata are formed and differentiated into wall and interior tissues, the apical pore region is recognizable, and multinucleate cells have formed near the base of the interior tissue. The multinucleate condition of certain cells within the

stroma must persist for four weeks if development is to continue. During this time the cells increase in size and number. The surrounding cells pull apart and begin to disintegrate. The cultures kept in cold for six weeks and then removed to room temperature are the first to show fusion nuclei in small asci after one week at higher temperature. In cultures retained in the cold room, fusion nuclei appear at nine or ten weeks. Although meiosis of the fusion nucleus and spore formation eventually occur in continuous low temperature, these events are hastened by higher temperature. After the formation of fusion nuclei there is no resting period in cultures kept at room temperature. Within two weeks the ascus enlarges, meiosis occurs, and spores are formed. However, cultures kept at low temperature continually have a second resting period of at least seven weeks before any increase in size of the young ascus occurs. Then, in rapid succession, the asci enlarge, meiosis occurs, and spores are formed.

The alternate periods of warmth and cold utilized in artificial culture are believed to be in force in nature as well. It is postulated that the conidia and ascospores reach the plant in spring and germinate in the ample moisture provided by melting snow. The developing vegetative hyphae become established in the plant tissues. Conidia are formed during the summer and serve to distribute the fungus to nearby plants. When the temperature drops nearly to freezing the formation of ascostromata is initiated. The fungus overwinters as ascostromata containing multinucleate cells or young asci with fusion nuclei. The following spring as the temperature slowly rises the snow cover melts. The asci mature and produce spores. These are liberated when new plants are developing rapidly. Conidia are also formed in the spring, on conidiophores arising from the apex of sterile ascostromata, and facilitate the spread of the fungus.

DEVELOPMENT OF THE ASCOSTROMATA

The ascospores of *M. tassiana* germinate within five hours on agar by the emergence of a hyaline papilla from either or both ends of a spore (FIG. 1). Conidia germinate in a similar manner. The papilla elongates into a broad germ tube, which as it enlarges develops septa and irregular branches (FIG. 2). Conidia are formed within three days (FIG. 16) and in their profusion color the colony a bright olivaceous green. Both ascospores (FIG. 15) and conidia (FIG. 17) are multinucleate before germinating. The individual cells of the mycelium are uninucleate except for the coenocytic hyphal tip (FIG. 2).



FIGS. 1–15. *Mycosphaerella tassiana*. 1. Germinating ascospore (after five hours). 2. Growing tip of hypha. 3. Ascocarp initial, composed of a compact mass of cells. 4. Ascogenous cells from locule of ascostroma (7 weeks). 5. Ascogenous cells giving rise to asci either directly or from croziers (two sections through one ascostroma). 6. Ascus prior to meiotic division. 7, 8, 9. Stages in first meiotic division of fusion nucleus in the ascus: 7, prophase; 8, anaphase; 9, late telophase. 10. Late anaphase, second meiotic division. 11. Four-nucleate ascus, all nuclei undergoing mitotic division. 12–15. Stages in maturation of ascospores: 12, each cell uninucleate; 13, each cell binucleate; 14, each cell containing two dividing nuclei; 15, mature spore, each cell four-nucleate. All × 1300.

In the colony the hyphae become entangled. Certain cells are enlarged, have a hyaline wall, and are filled with granular cytoplasm. In these cells, cleavage of cytoplasm and wall formation produce a multicellular structure, the ascocarp initial (FIG. 3). This structure enlarges greatly and becomes differentiated into outer wall, locule, and pore area. All cells are uninucleate at this stage. After the ascostroma reaches full size the walls of the outer cells become thickened and dark brown.

Certain cells toward the base of the locule enlarge slightly and become multinucleate and deeply staining. These are the ascogenous cells (FIG. 4). They are variable in size and shape. Five to fourteen develop in a locule, each containing two to five nuclei. Ascus formation begins with the protrusion of an upright segment from one of the ascogenous cells (FIG. 5). This segment may become bent, crozier-like, or it may merely enlarge. Two nuclei migrate into the segment and a septum forms across the base. The nuclei then fuse, and the fusion nucleus and young ascus enlarge. The asci arise from the compact group of ascogenous cells and spread out into the locule from a common base. The space is created by disintegration of the small cells of the locule. Any remnants of these cells are compressed against the inner wall of the locule at maturity. No paraphysis-like structures are present between the asci. The young fusion nucleus within the ascus is about 1 μ in diameter. It enlarges to 5–10 μ in diameter before meiosis with the deeply stained nucleolus 2–3 μ in diameter. The ascus is filled with granular cytoplasm and finally becomes surrounded by a nonstaining, gelatinous wall 1.5–2 μ thick (FIG. 6).

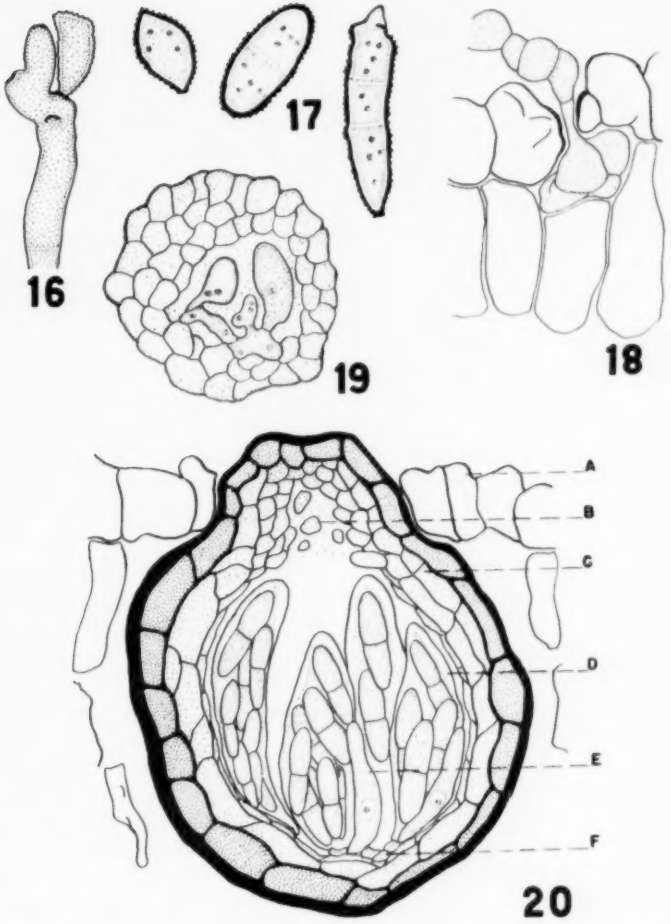
Observation of meiotic stages is difficult because of the minute size of chromosomes in this fungus. In the metabolic nucleus, shortly before meiotic division, the chromatin is visible as dots. The chromatin begins to clump and to form twisted strands (FIG. 7). As the chromosomes shorten and thicken, pair and separate, eight are distinguishable (FIG. 8). By this time the nucleolus is no longer visible. Later, as the two groups of chromosomes clump at opposite ends of the nuclear area, two nucleoli appear (FIG. 9). The two sets of chromosomes, each with its nucleolus, move apart in the ascus to form two nuclei. The second meiotic division (FIG. 10, anaphase) produces four haploid daughter nuclei, each containing four chromosomes. A mitotic division then takes place (FIG. 11). Coincident with the production of eight nuclei a delicate membrane is formed delimiting a single nucleus and the surrounding cytoplasm. The spore initials become binucleate, the two nuclei move to opposite ends of the spore, and a septum forms across the middle (FIG. 12). Two additional nuclear divisions occur in the spore (FIGS.

13, 14), so that at maturity each cell of the spore is quadrinucleate (FIG. 15). A distinct epispore surrounds the spore.

In *Mycosphaerella typhae* several differences were noted in cultural characteristics. No conidia are formed. The colonies remain relatively small and dense. Ascocarp initials are produced in the colonies within four to five days. Stromatic knots which form within the medium are almost always sterile, while those produced in the mat of aerial mycelium develop to maturity. In sterilized leaf material vegetative mycelium is produced in smaller quantity and stromatic development is more readily observed. The ascostromata mature at room temperature within fourteen days, in marked contrast to those of *M. tassiana* (see TABLE 1).

As in *M. tassiana,* the ascostroma initial develops from enlargement and division of a single cell of the hypha (FIG. 18). The stroma enlarges and becomes differentiated into wall, locule and pore area. At the base of the locule one or two deeply stained, multinucleate cells arise. Later four to nine are visible. The ascogenous cells produce young asci (FIG. 19), at first binucleate, later containing a single fusion nucleus. As the asci enlarge, they crush the remaining locule cells against the inner wall and completely fill the cavity. The fusion nucleus enlarges in the ascus until the nucleolus reaches a diameter of 1.5 μ and the nuclear membrane 2–5 μ. No meiotic or mitotic stages were observed in this species. Only young asci containing a large fusion nucleus and older asci with spores already formed could be found. The spores become one-septate, each cell containing a single nucleus and two oil droplets.

Although they differ in cultural characteristics, *M. tassiana* and *M. typhae* are similar morphologically. They differ in size and in distribution, *M. tassiana* occurring chiefly in arctic and alpine regions and *M. typhae* in temperate regions. FIG. 20, a section through a mature ascostroma of *M. typhae,* illustrates the morphology of both species. The wall (C) of the ascostroma (immersed in the leaf tissue, A) is surrounded by a dark outer layer and is composed of two to three layers of more or less elongated cells. The pore area (B) is partially filled with disintegrating cells. Within the locule, the fascicle of asci (D, E) arises from the ascogenous cells (F). In almost all ascostromata sectioned, asci may be found in several stages of development. At maturity, the spores lie crowded in the saccate ascus. As the ascus pushes upward in the locule, the cells of the pore area are compressed or disintegrate, leaving a passage to the exterior. A mature ascus, elongated by increased osmotic pressure of the contents, protrudes through



FIGS. 16, 17. *Mycosphaerella tassiana*. 16. Conidiophore with attached conidium. 17. Maturing conidia. FIGS. 18–20. *Mycosphaerella typhae*. 18. Beginning of cell division to form ascocarp initial after hyphal tip penetrated stomate. 19. Section through locule of ascostroma showing ascogenous cells and young asci in cavity of locule. 20. Section through mature ascostroma (explanation in text). All × 1300.

the apical pore. The outer wall of the ascus breaks and the spores emerge surrounded by the gelatinous inner wall layer.

DISCUSSION

Mycosphaerella tassiana and *M. typhae* are placed in the Loculoascomycetes because of the development of a vegetative stroma, the formation of ascogenous cells within the stroma, and the double-walled asci. Locule formation and the development of asci in a fascicle which occupies the entire locule are in accordance with the "*Dothidea* developmental type" described by Luttrell (**1951**). The order Dothideales contains fungi having such a type of development. Within this order both uni- and pluriloculate, immersed forms are placed in the family Dothideaceae by Luttrell (**1951**). Miller (**1949**) separated the uniloculate forms into the family Mycosphaerellaceae, and retained the Dothideaceae for pluriloculate forms. He admitted, however, that there were transition forms in the genus *Mycosphaerella* which showed the aggregation of ascostromata into a compound stroma with numerous locules. *M. typhae* frequently exhibits this condition, with the ascostromata aggregated into elongated groups containing many locules. *M. tassiana* also forms grouped ascostromata, at times entirely united by the stromatic crust. Thus it appears that in *Mycosphaerella* there is a series which grades from separate ascostromata to aggregated ascostromata and finally to the usual concept of dothideaceous forms with numerous locules in a massive stroma. It seems to me that the genus *Mycosphaerella* is best regarded as a member of the Dothideaceae in the modern sense, rather than being separated in the family Mycosphaerellaceae.

Mycosphaerella tassiana and *M. typhae* are both members of the subgenus *Didymellina* (von Arx, **1949**). The development of a number of species of *Mycosphaerella,* chiefly in the subgenus *Mycosphaerella,* has been studied previously. One striking difference is noted in reading the descriptions of, for example, *M. tulipiferae* (Schw.) Higgins (**1936**). In this species, as well as in several others, the microconidia fertilized the ascogonium to produce the binucleate condition. Neither trichogynes nor microconidia were found to be involved in the life cycles of *M. tassiana* and *M. typhae,* and no fertilization occurred with a nucleus from the outside. It has been impossible to answer with certainty the question as to the origin of the ascogenous cells. They are visible at an early stage in differentiation of the ascostroma. There is no evidence of plasmogamy preceding the formation of ascogenous cells, as suggested by Luttrell (**1953**) for *Glonium stellatum* and Shoemaker

(**1955**) for *Cochliobolus sativus*. It appears that any cell of the locule may enlarge and may become an ascogenous cell. In the process, the cytoplasmic content increases and the nucleus divides mitotically. After the cell has further increased in size nuclear division results in a large, multinucleate ascogenous cell, frequently irregular or lobed in outline. The theory that "favored" cells in the locule attain some physiological advantage and become ascogenous cells has been propounded by Wehmeyer (**1954**, **1955a**, **1955b**). The nature of the mechanism which directs the formation of ascogenous cells is still unknown.

NOMENCLATURE

On the bases of host plant or minor variations in morphology, numerous species have been described which differ but little from *Mycosphaerella tassiana*. Multiplication of names on the basis of substrate was first discussed and condemned by Lind (**1926**). At that time he listed several names as synonymous with *M. tassiana*. Petrak (**1940**, **1942**, **1944**, **1947**, **1952**) studied some type specimens and found that they were merely forms of *M. tassiana*. Wehmeyer (**1946**) also suggested that certain species were nothing but growth forms of *M. tassiana*. In 1949 von Arx gave a comprehensive list of names which he considered as synonymous with *M. tassiana*. He studied original or authentic material of thirty-two taxa and, from the descriptions and figures of seventeen other species for which no authentic material was available, placed them all in synonymy with *M. tassiana*. According to the descriptions, it is also probable that numerous additional species are identical with this fungus. At least for the present, *Mycosphaerella tassiana* (de Not.) Johans. is accepted as the valid name for this common circumpolar species.

The conidial stage of *M. tassiana*, briefly mentioned in the cultural study, has rarely been reported as a part of the life cycle of the fungus. Karsten (**1872**) associated *M. tassiana* with *Cladosporium graminum* Link. Von Arx (**1949**) obtained conidia, which he reported as *Cladosporium herbarum* Link ex Fries, in cultures from only two isolates of *M. tassiana*. In 1950, however, he designated *M. tulasnei* (Jancz.) Lindau as a synonym of *M. tassiana*. *M. tulasnei* had previously been reported as the ascigerous stage of *Cladosporium herbarum* by Janczewski (see Sorauer, **1894**); this was later confirmed by Ruehle (**1931**). Kienholz (**1944**) has also reported obtaining both *Cladosporium herbarum* and *M. tulasnei* in culture from isolates of overwintered apple and pear leaf material in Oregon. Von Arx (**1950**) obtained the conidial stage in culture from several strains of *M. tassiana* (*M. tulasnei*)

collected in the Swiss Tyrol. Bisby and Mason (**1940**) recorded *C. herbarum* as the imperfect stage of *M. tulasnei* from Britain. Wehmeyer (**1946**) reported the conidial stage accompanying ascostromata of *M. tassiana* on herbaceous stems and leaves from Wyoming. He referred the conidial fungus to the form genus *Scolecotrichum*.

In nature the conidial stage appears to belong to *Scolecotrichum*, but the conidia are finely echinulate rather than smooth. The infrequent formation of conidial chains and the roughened walls of the conidia would remove it from *Cladosporium*. It differs from *Heterosporium* in having simple or one-septate instead of three- to many-septate conidia. In culture, the fungus produces chains of conidia as well as single conidia, as does the *Hormodendron* form of *Cladosporium* (de Vries, **1952**). The conidia, however, are frequently more than one-septate and are always echinulate at maturity, and are then more in accordance with the concept of *Heterosporium* than of *Cladosporium*.

De Vries (**1952**) united the form genera *Cladosporium* and *Heterosporium* under the former name. He stated that the perfect stage, when present, is *Mycosphaerella*. The genus *Cladosporium*, as delimited by him, is comprehensive enough to include the fungus under discussion both in its natural state and in culture. Different strains of the imperfect stage of *M. tassiana* may be considered either as *C. herbarum* or as *C. macrocarpum*, as delimited by de Vries (**1952**) chiefly on the basis of spore size. It seems probable that the two species could be united under *C. herbarum*, the earlier name. The variable *M. tassiana* would then have as its imperfect stage *C. herbarum* Link ex Fries, which also includes a wide range of variation.

The name *Mycosphaerella typhae* (Lasch) Lindau has received two interpretations throughout the years since the fungus was first described. The original description of *Sphaeria typhae* included no microscopic characteristics, but merely stated "Lanceolata v. linearis, perithec. cohaerentibus subrotundis intus albonigrescentibus" (Lasch in Rabenhorst, **1845**). Fuckel (**1870**) transferred the species to *Sphaerella* and described the spores as hyaline, oblong-clavate, and $10 \times 4\ \mu$. Auerswald (**1869**) also referred it to the genus *Sphaerella*, but he described the spores as fuscous, cuneate-oblong, and $14 \times 5\ \mu$. The two earliest descriptions of the microscopic characters of *Sphaerella typhae* thus refer to two species belonging to different genera. The confusion of two fungi under one name by these authors has been perpetuated by later mycologists.

Study of isotype material obtained from the Farlow Herbarium (Rab.-Klotzsch. Herb. Myc. no. 660) has clarified the situation. The

fungus present on this material is the *Mycosphaerella*. The ascostromata are grouped in longitudinal rows between the leaf veins and extend up to 3 mm in length. The locules measure 48–80 μ in diameter. The asci are few, oblong to saccate, sessile, with a double wall thickened toward the apex. They are 28–38.5 × 10.5–14 μ. The spores are greenish-hyaline, clavate to cuneate with the upper cell broader, slightly constricted at the septum, and 12.5–14.5 × 4–5 μ. The fungus is identical with the Michigan specimens. The species is correctly cited as *Mycosphaerella typhae* (Lasch) Lindau, and the brown-spored fungus is a distinct entity. The names *Sphaerella typhae* of Auerswald (**1869**) and *Phaeosphaerella typhae* of Petrak (**1925**) are synonyms of *Sphaeria typhae* Lasch. The specimens cited were misdeterminations of Lasch's species and must therefore receive a new name in the genus *Phaeosphaerella* (or *Venturia*, see Korf, **1956**) to which they belong.

SUMMARY

The life histories of two species of *Mycosphaerella*, *M. tassiana* (de Not.) Johans. and *M. typhae* (Lasch) Lindau, were studied in culture. *M. tassiana* produces a conidial stage, whereas *M. typhae* does not. They are in many respects similar in development and in morphology. Both are members of the subgenus *Didymellina* of *Mycosphaerella*. They belong, in modern classification, to the order Dothideales, family Dothideaceae of the Loculoascomycetes.

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THE AGARIC FLORA OF TEXAS. II. NEW TAXA OF WHITE- AND PINK-SPORED AGARICS [1, 2]

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This paper is the second of a series on new agarics discovered in eastern Texas. The species described in the first report (**4**) were all white-spored, and, in the scheme of modern taxonomy, belong to the families Russulaceae, Amanitaceae and Agaricaceae sensu Singer (**1**), as well as to the Boletaceae. This paper presents descriptions of new species and varieties of white-spored agarics belonging to the Tricholomataceae and a new species of *Rhodophyllus* belonging to the Rhodophyllaceae.

The type collections have been deposited in the herbarium of the University of Michigan, Ann Arbor, Michigan.

Marasmius puniceus sp. nov.

Pileus 1–3.5 mm latus, convexus vel plano-convexus, siccus, glaber, "eugenia red." [3] Lamellae distantes, "Ventian pink" vel "flesh pink," postice in collarium connatae. Stipes 1.5–2.5 cm longus, 0.2–0.3 mm crassus, corneus, nitidus, glaber, "eugenia red" demum basi "Vandyke red" vel "madder red." Sporae ovato-oblongae vel cylindricae, non-amyloideae, 10–12.5 × 2–3.1 μ; pleurocystidia clavata vel subfusoidea; cheilocystidia clavata, ramosa ad apicem; cellulae cuticulae clavatae, echinulatae.

Pileus 1–3.5 mm broad, convex becoming plano-convex or unchanging with age, margin entire, incurved, becoming decurved, surface dry, dull, glabrous, sulcate, evenly colored bright red ("eugenia red") during all stages of development.

Flesh very thin (c. 1 mm), pliant, membranous, concolorous with the surface, taste and odor not distinctive.

Lamellae distant, thick with obtuse edges, forming a collar at the base, narrow, pink ("Venetian pink" to "flesh pink") when mature,

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[2] Grateful acknowledgment is made to Dr. A. H. Smith for his continued assistance and guidance in this study. Appreciation is also expressed to my wife, Ellen Thiers, for preparation of the Latin diagnoses.

[3] All colors given in quotations are from Ridgway, Color Standards and Color Nomenclature. Washington, D. C. 1912.

margin entire, concolorous with the gill faces; lamellulae irregularly dispersed.

Stipe 1.5–2.5 cm long, 0.2–0.3 mm broad at the apex, inserted, tapering slightly toward the apex, solid, surface polished, glabrous, dry, red ("eugenia red") when young, changing to dark red ("Vandyke red" to "madder red") toward the base when older.

Spores hyaline in KOH, non-amyloid, oblong-lanceolate to nearly cylindric, smooth, thin-walled, 10–12.5 × 2–3.1 μ; basidia clavate, 4-spored, 35–40 × 9–11 μ; pleurocystidia scattered, clavate to subfusoid, hyaline, thin-walled, 23–38 × 7–9 μ; cheilocystidia numerous, obscurely clavate with many short knobs or branches at the apex, hyaline, thin-walled, 18–30 × 8–10 μ; gill trama composed of interwoven to occasionally parallel, hyaline, filamentous hyphae c. 4 μ in diam.; pileus trama composed of compactly interwoven, hyaline, filamentous hyphae c. 5 μ in diam.; cuticle differentiated as a compact layer of hyaline "broom cells"; clamp connections present.

Gregarious to subcespitose on old cow dung and debris. Oak hardwoods near Millican, Brazos Co., Texas, May 16, 1953, *H. D. Thiers 1846*, TYPE.

The bright red pileus, red stipe, densely gregarious to cespitose fruiting habit and relatively small spores distinguish this marasmius. It fruits on dung but not exclusively so, as does *Marasmius coprophila*. It is probably most closely related to this latter species but is easily distinguished by the characters mentioned above. It also seems to be close to the species-complex of *M. helvolus*, *M. haematocephalus* and *M. tageticolor* but the spores of those species are much larger. Singer (2) described *M. tucumanus* from Argentina, to which this species also appears to be related.

Mycena radicata sp. nov.

Pileus 0.8–1 cm latus, convexus demum plano-convexus, udus, glaber, "sepia" demum "Saccardo's umber." Lamellae adnatae, albae, subdistantes. Stipes 2.5–3 cm longus, 0.8–1 mm crassus, aequalis, radicatus, glaber, albus. Sporae amyloideae, globosae vel subglobosae, 7–7.8 × 6.3–6.8 μ; pleurocystidia subcylindrica vel fusoideo-ventricosa; cheilocystidia pleurocystidiis similia.

Pileus 0.8–1 cm broad, convex becoming plano-convex, margin appressed to the stipe becoming straight, entire becoming eroded, surface moist, glabrous, obscurely striate near the margin, when young dark brown ("sepia") on the disc, becoming slightly paler on the margin, when older fading to umber ("Saccardo's umber").

Flesh thin (1–2 mm), white, cuticle concolorous with the surface, taste slightly bitter, odor not distinctive.

Lamellae ascending adnate, thin, narrow, subdistant, white during all stages of development, margin entire, concolorous with the gill faces; lamellulae of 1–3 lengths.

Stipe 2.5–3 cm long, 0.8–1 mm broad at the apex, equal, rooting with a well developed pseudorhiza 2–2.5 cm in length, stuffed becoming hollow, surface glabrous, moist, white-trunslucent during all stages of development.

Spores hyaline in KOH, weakly amyloid, smooth, thin-walled, globose to subglobose to nearly oblong, 7–7.8 × 6.3–6.8 μ, with a long, conspicuous apiculus; basidia narrowly clavate, 2- and 4-spored, 19–23 × 7–9 μ; pleurocystidia numerous, subcylindric to obscurely fusoid-ventricose, hyaline, moderately thick-walled, 43–72 × 9–14 μ; cheilocystidia numerous, cylindric to clavate to fusoid-ventricose, hyaline, thin-walled, 29–48 × 7–11 μ; gill trama composed of hyaline, thin-walled hyphae with irregularly shaped cells; pileus trama interwoven, with a well differentiated hypoderm; cuticle poorly differentiated as a narrow layer of interwoven hyphae; examined for clamp connections but none found.

Scattered in humus near decayed hardwood log. Pine-hardwood forest near Richards, Montgomery Co., Texas, April 2, 1953, *H. D. Thiers 1778*, TYPE.

This species, belonging to the section Typicae of the subgenus Eumycena, is distinct because of the definite pseudorhiza, white stipe and combination of large, conspicuous pleurocystidia and relatively large, globose spores. It appears most closely related to *Mycena supina*, from which it is most easily distinguished by the smooth-walled cheilocystidia. The amyloid spores separate it from *M. floccipes*.

Mycena avellaneibrunnea sp. nov.

Pileus 1.5–2 cm latus, conicûs demum subconicus vel convexus, siccus, glaber, "cinnamon drab" vel "benzo brown" demum "light cinnamon drab" vel "wood brown." Lamellae subdistantes, adnatae, angustae, "tilleul buff" vel "pale pinkish buff." Stipes 4–6 cm longus, 1–1.5 mm crassus, aequalis, siccus, glaber, sursum "avellaneous" deorsum "wood brown." Sporae amyloideae, oblongae vel ovato-oblongae, 10.7–12.4 × 6–6.7 μ; pleurocystidia nulla; cheilocystidia subclavata vel fusoideo-ventricosa.

Pileus 1.5–2 cm broad, conic becoming subconic to acutely convex, margin appressed when young becoming straight with age, entire, surface dry, glabrous, striate to the disc, when young dark gray to violaceous brown ("cinnamon drab" to "benzo brown") fading to gray to brown ("light cinnamon drab" to "wood brown") when older.

Flesh thin (1–2 mm), concolorous with the surface, taste slightly bitter, farinaceous, odor not distinctive.

Lamellae subdistant, ascending adnate, narrow, thin, pallid to buff

("tilleul buff" to "pale pinkish buff"), margin entire, concolorous with the gill faces; lamellulae of 2–3 lengths.

Spores hyaline in KOH, amyloid, oblong to oblong-lanceolate, smooth, thin-walled, 10.7–12.4 × 6–6.7 μ; basidia 2- and 4-spored, narrowly clavate, 18–21 × 7–8 μ; pleurocystidia not differentiated; cheilocystidia scattered, hyaline, thin-walled, subclavate to fusoid-ventricose with long, flexuous apices, 23–62 × 6–12 μ; gill trama composed of hyaline, filamentous interwoven hyphae c. 7 μ in diam.; pileus trama composed of hyphae with irregularly shaped cells; cuticle differentiated as a layer c. 15 μ broad of hyaline, incrusted hyphae c. 2 μ in diam.; examined for clamp connections but none found.

Gregarious on decayed hardwood log in dense pine-hardwood forest. Sam Houston National Forest near Coldspring, San Jacinto Co., Texas, May 20, 1952, *H. D. Thiers 1824*, TYPE.

This mycena is characterized by relatively large carpophores, smooth pileus, large spores, no pleurocystidia and large, smooth cheilocystidia. It is somewhat a parallel of *Mycena galericulata* but with smooth cheilocystidia.

MYCENA EPIPTERYGIA (FRIES) KUMMER VAR. **cespitosa** var. nov.

Dense gregaria vel cespitosa. Pileus centro "Dresden brown" vel "Prout's brown" vel "mummy brown," margine "pinard yellow."

Pileus 0.8–2 cm broad, convex becoming broadly convex, margin straight, appressed to the stipe, entire becoming eroded, surface glabrous, moist, viscid when wet, olive brown ("Dresden brown" to "Prout's brown" to "mummy brown") toward the disc, changing to yellow ("pinard yellow") toward the margin, not fading with age.

Flesh thin (1–2 mm), white, taste pungent to farinaceous, odor slightly farinaceous.

Lamellae ascending adnate with a short decurrent tooth, narrow, close to subdistant, white to pale yellow, margin entire, concolorous with the gill faces; lamellulae of two lengths.

Stipe 3–5 cm long, 1.5–2.5 mm broad at the apex, equal, stuffed becoming hollow, surface glabrous, viscid, yellow ("pinard yellow") during all stages of development.

Spores hyaline in KOH, amyloid, smooth, thin-walled, broadly ellipsoid to ovoid, 9.3–10.5 × 6.2–7.8 μ; basidia clavate, 4-spored, 31–38 × 7–9 μ; pleurocystidia not differentiated; cheilocystidia numerous, hyaline, thin-walled, clavate to broadly clavate with numerous short, rod-like projections or with few branches or unbranched, 30–38 × 6–9 μ; gill trama composed of interwoven to subparallel, hyaline, filamentous hyphae c. 9 μ in diam.; gill edge gelatinizing in KOH; pileus trama interwoven,

composed of large (c. 8 μ), filamentous hyphae; cuticle differentiated as a broad (c. 150 μ), layer of hyaline, interwoven hyphae, gelatinizing in KOH, surmounting a hypoderm (c. 40 μ) of closely interwoven, filamentous hyphae; clamp connections present.

Densely gregarious to cespitose at base of conifer stump in mixed forest. Sam Houston National Forest near Richards, Montgomery Co., Texas, Dec. 31, 1951, *H. D. Thiers 1433*, TYPE.

This variety is distinguished by the cespitose manner of fruiting and the presence of branched or unbranched cheilocystidia. The cheilocystidia are somewhat similar to those in *Mycena griseoviridis* and probably indicate a close relationship between the two species.

Collybia lignicola sp. nov.

Pileus 4–8 cm latus, convexus demum late convexus vel plano-convexus, siccus, glaber, "pinkish buff" vel "light buff," maculatus "army brown" vel "natal brown." Lamellae adnatae vel adnexae, confertae, angustae, "pale pinkish buff" vel "pinkish buff." Stipes 5–7 cm longus, 5–12 mm crassus, siccus, pruinosus, glabrescens, "pale pinkish buff," tactu "bister." Sporae subglobosae vel ovoideae, 7.8–9.3 × 6.5–7.5 μ; pleurocystidia nulla; cheilocystidia fusoidea vel subfusoidea; pilocystidia cheilocystidiis similia.

Pileus 4–8 cm broad, convex becoming broadly convex to plano-convex, margin incurved becoming broadly decurved with age, entire, surface dry, dull, glabrous, buff ("pinkish buff" to "light buff") during all stages of development, brown ("army brown" to "natal brown") spots irregularly dispersed over the surface.

Flesh thin (2–4 mm), tough, concolorous with the surface, taste bitter, odor slightly aromatic.

Lamellae adnate to shallowly adnexed, close to crowded, very narrow, thin, forking irregularly throughout, evenly buff, ("pale pinkish buff" to "pinkish buff") when mature, margin entire, concolorous with the gill faces; lamellulae of 2–3 lengths.

Stipe 5–7 cm long, 5–12 mm broad at the apex, tapering toward the base, stuffed to hollow, surface dry, ridged, pruinose, glabrescent, buff ("pale pinkish buff") at maturity, dark brown ("bister") stains when bruised.

Spores hyaline in KOH, non-amyloid, subglobose to ovoid, smooth, thin-walled, 7.8–9.3 × 6.5–7.5 μ; basidia clavate, 4-spored, 17–25 × 8–11 μ; pleurocystidia not differentiated; cheilocystidia abundant, forming a sterile margin, hyaline, thin-walled, fusoid to aciculate to obscurely fusoid-ventricose with narrowed, somewhat elongated apices, 34–42 × 6–12 μ; gill trama composed of interwoven to subparallel, hyaline, filamentous hyphae c. 4 μ in diam.; pileus trama composed of hyaline, interwoven, filamentous hyphae c. 6 μ in diam.; cuticle differentiated as

a layer of interwoven hyphae with occasional pilocystidia similar to the cheilocystidia, 10–18 × 8–10 μ; examined for clamp connections but none found.

Subcespitose on pine log in pine-hardwood forest. Sam Houston National Forest near Coldspring, San Jacinto Co., Texas, May 23, 1953, *H. D. Thiers 1909*, TYPE.

This species resembles *Collybia maculata* but the spores are larger, the cheilocystidia are differently shaped, and pilocystidia are present. It also appears to be closely related to an undescribed species collected by Smith in Tennessee; there are, however, differences in fruiting habit, stipe, taste and odor as well as in spore size.

Collybia delicata sp. nov.

Pileus 4–9 cm latus, late conicus vel convexus demum planus vel umbonatus, siccus, glaber, "pale pinkish buff" vel "sayal brown" demum "warm sepia." Lamellae subdistantes vel distantes, adnatae vel adnexae, "pale ochraceous buff" vel "light ochraceous buff." Stipes 4–6 cm longus, 5–9 mm crassus, aequalis, pruinosus, siccus, "cartridge buff" demum ad apicem "deep dull lavender." Sporae ovoideae vel subellipsoideae, 6.5–7.8 × 4–4.7 μ; pleurocystidia cylindrica vel fusoidea et late clavata; cheilocystidia fusoideo-ventricosa et cylindrica vel filamentosa; caulocystidia cylindrica vel filamentosa vel ventricosa; cellulae cuticulae subglobosae.

Pileus 4–9 cm broad, broadly conic to convex becoming plane to umbonate with a short, obtuse umbo, margin obscurely striate to striate to plicate-striate, plane to slightly decurved, entire becoming eroded, surface dry, glabrous, often obscurely areolate on the disc with age, when young evenly buff ("pale pinkish buff") except brown ("sayal brown") on the umbo, with age the margin frequently becoming dark brown ("warm sepia").

Flesh thin (3–4 mm), submembranous, white, taste slightly bitter, odor not distinctive.

Lamellae subdistant to distant, adnate to adnexed with a short decurrent tooth, broad, thin, buff ("pale ochraceous buff" to "light ochraceous buff") when mature, margin entire, concolorous with the gill faces; lamellulae of three lengths.

Stipe 4–6 cm long, 5–9 mm broad at the apex, round to flattened, equal except for the bulbous enlargement at the base, solid becoming hollow, surface pruinose, longitudinally striate, dry, sometimes floccose at the base, when young pale buff ("cartridge buff"), the apex changing to lavender ("deep dull lavender") when older, base unchanging.

Spores hyaline in KOH, non-amyloid, smooth, thin-walled, ovoid to subellipsoid, 6.5–7.8 × 4–4.7 μ; basidia clavate, 4-spored, 30–36 × 7–9 μ; pleurocystidia of two types: one type abundant, hyaline, moderately thick-walled, frequently attached to lactifers in the gill trama, cylindric

to fusoid, tapering to an acute to subacute apex, 54–78 × 8–12 μ; second type scattered, more common toward the gill edge, broadly clavate with an abruptly narrowed, somewhat elongated apex, hyaline, thin-walled, 29–39 × 7–9 μ; cheilocystidia of two types: one type similar to the pleurocystidia except sometimes fusoid-ventricose, numerous, hyaline, 37–45 × 14–16 μ; second type nearly cylindric to filamentous with obtuse apices, hyaline, thin-walled, 31–45 × 6–8 μ; gill trama hyaline, interwoven, composed of filamentous hyphae c. 6 μ in diam.; pileus trama composed of compactly interwoven, hyaline, filamentous hyphae c. 7 μ in diam.; cuticle differentiated as a closely packed layer of hyaline, thin-walled, pyriform cells; caulocystidia scattered to numerous, hyaline, thin-walled, cylindric to filamentous to obscurely ventricose, 40–53 × 4–6 μ; clamp connections present.

Gregarious to subcespitose in humus under oaks. Hardwoods near the clay pits, Wellborn, Brazos Co., Texas, June 9, 1952, *H. D. Thiers 1684*, TYPE.

This mushroom has the general appearance and stature of *Collybia glatfelteri* or *C. tenuifolia* and is probably related to them, but it has several characters which make it distinct. The presence of two types of cheilocystidia and pleurocystidia, the presence of caulocystidia and differently sized spores are all characters which separate it from either of the two species mentioned above.

Tricholomopsis squamosa sp. nov.

Pileus 3.5–4.5 cm latus, conicus demum plano-convexus vel planus, siccus, fibrillosus demum squamosus, margine "Hay's russet" vel "russet" demum "Mars brown," centro "tawny" demum "ochraceous tawny." Lamellae sinuatae vel adnexae, confertae vel subdistantes, "avellaneous" demum "light buff" vel "warm buff." Stipes 2–3.5 cm longus, 5–8 mm crassus, aequalis, "Mikado brown" demum "cinnamon buff," siccus, fibrillosus. Sporae non-amyloideae, ovoideae vel subglobosae, 9.3–12 × 7.5–8.5 μ; pleurocystidia nulla; cheilocystidia fusoidea vel clavata vel fusoideo-ventricosa.

Pileus 3.5–4.5 cm broad, obtusely conic becoming plano-convex to plane with an irregularly uplifted margin, margin incurved when young, entire to eroded, surface dry, densely covered with fibrils which become aggregated into squarrose scales, reddish ("tawny") toward the disc and red ("Hay's russet" to "russet") toward the margin when young, reddish to reddish brown ("tawny" to "ochraceous tawny") on the disc and brown ("Mars brown") toward the margin when older.

Flesh thin (2–3 mm), white, unchanging when bruised or exposed, taste bitter, odor not distinctive.

Lamellae sinuate to adnexed, close to subdistant, narrow, lavender-gray ("avellaneous") when young, becoming yellowish ("light buff" to

"warm buff") when older, margin entire, concolorous with the gill faces; lamellulae of 2–3 lengths.

Stipe 2–3.5 cm long, 5–8 mm broad at the apex, equal, solid, brown ("Mikado brown") when young, becoming buff ("cinnamon buff") when older, surface dry, appressed fibrillose.

Spores hyaline in KOH, non-amyloid, smooth, thin-walled, ovoid to subglobose, 9.3–12 × 7.5–8.5 μ; basidia narrowly clavate, apparently 1-, 2- and 4-spored, sterigmata of unequal lengths, 28–37 × 7–9 μ; pleurocystidia not differentiated; cheilocystidia abundant, forming a sterile margin, thin-walled, hyaline, broadly fusoid to clavate with a submucronate apex to obscurely fusoid-ventricose, 30–46 × 12–18 μ; gill trama parallel to interwoven, composed of thin-walled, hyaline, filamentous hyphae c. 8 μ in diam.; pileus trama loosely interwoven, composed of large (c. 12 μ), thin-walled, hyaline, filamentous hyphae; cuticle differentiated as a layer of closely packed, radially arranged, filamentous hyphae c. 9 μ in diam., staining reddish brown in KOH; pilocystidia not seen; clamp connections present.

Gregarious on dead hardwood stump in mixed woods. Sam Houston National Forest near Huntsville, Walker Co., Texas, June 10, 1952, *H. D. Thiers 1709*, TYPE.

This tricholomopsis is probably closely related to *T. rutilans*, but is distinct in several ways. The pileus is differently colored and is fibrillose-scaly; the lamellae are not bright yellow; the stipe is buff to dark brown; the spores are much larger and the cheilocystidia much smaller. It is distinct from *T. variegata* by the white lamellae and the presence of cheilocystidia in great abundance.

MELANOLEUCA REAI Singer var. **texana** var. nov.

Pileus 5–10 cm latus, sapor amarus; sporae 6.5–9 × 5.8–6.5 μ. Stipes centralis.

Pileus 5–10 cm broad, broadly convex becoming plane to planoconvex, occasionally broadly umbonate, margin entire, incurved when young becoming decurved to nearly plane with age, surface smooth, glabrous, appearing water-soaked, dark yellow to brown ("Isabella color" to "Dresden brown") when young, paler on the disc, becoming evenly tan ("warm buff") with age.

Flesh thick (3–5 mm) on the disc becoming thinner toward the margin, white, floccose; taste bitter, odor not distinctive.

Lamellae adnate with a short decurrent tooth, white during all stages of development, close to subdistant, thin, narrow, margin entire, concolorous with the gill faces; lamellulae of 2–3 lengths.

Stipe 5–6.5 cm long, 4–8 mm broad at the apex, often flattened toward the apex, stuffed, equal to tapering slightly toward the apex, base

usually bulbous, surface moist, glabrous to fibrillose, white to buff ("pale buff").

Spores cream color in mass, hyaline in KOH, strongly amyloid, punctate to verrucose, ovoid to subellipsoid, walls slightly thickened, 6.5–8.8 × 5.8–6.5 μ; basidia clavate, 4-spored, 30–36 × 9–14 μ; pleurocystidia not differentiated; cheilocystidia scattered, hyaline, thin-walled, inconspicuous, ventricose with elongated, tapering, subacute apices, 33–40 × 6–11 μ; gill trama composed of interwoven, hyaline, filamentous hyphae c. 6 μ in diam.; pileus trama composed of lossely interwoven, hyaline, filamentous hyphae c. 10 μ in diam.; cyticle interwoven, not strongly differentiated; examined for clamp connections but none found.

Forming a large fairy ring in open area. Texas Agricultural and Mechanical College Campus, College Station, Brazos Co., Texas, September 4, 1953, *H. D. Thiers 1913*, TYPE.

This variety is distinct from the more common one found in the United States in that it has a decidedly bitter taste, larger pileus and slightly smaller spores. Smith and Rea (**3**) observed a slightly eccentric stipe in California material which was not seen in the local collections.

Rhodophyllus verrucosus sp. nov.

Pileus 1.5–2.5 cm latus, convexus demum plano-convexus vel late convexus, hygrophanus, udus, glaber, "snuff brown" demum "sayal brown" vel "tawny olive." Lamellae confertae vel subdistantes, decurrentes, "pale pinkish buff" demum "light pinkish cinnamon." Stipes 2–3 cm longus, 2–3 mm crassus, aequalis, "drab" udus, glaber, pruinosus ad apicem. Sporae fusiformae vel ventricosae, verrucosae, 12.5–15.5 × 6.2–7.8 μ; cystidia nulla.

Pileus 1.5–2.5 cm broad, convex becoming plano-convex to broadly convex, margin slightly incurved when young, becoming decurved to plane with age, entire, hygrophanous, surface moist, even, glabrous, when young brown ("snuff brown" to "bister") changing to light brown to olivaceous ("sayal brown" to "tawny olive") with age.

Flesh thin (2–4 mm), firm, concolorous with the surface; taste and odor not distinctive.

Lamellae close to subdistant, decurrent, thin, narrow, pinkish tan ("pale pinkish buff") when young changing to pink ("light pinkish cinnamon") with age, margin entire, concolorous with the gill faces; lamellulae of 2–3 lengths.

Stipe 2–3 cm long, 2–3 mm broad at the apex, equal, stuffed becoming hollow with age, gray ("drab") during all stages of development, surface moist, glabrous except pruinose near the apex.

Spores pink in mass, hyaline to pale straw color in KOH, nodulose to warty but not strongly angular, fusiform to ventricose in outline,

12.5–15.5 × 6.2–7.8 μ; basidia clavate, 4-spored, 28–33 × 8–10 μ; pleurocystidia and cheilocystidia not differentiated; gill trama composed of hyaline, interwoven, filamentous hyphae c. 6 μ in diam.; pileus trama composed of intricately interwoven, hyaline, filamentous hyphae c. 7 μ in diam.; cuticle poorly differentiated as a compact layer of interwoven hyphae c. 5 μ in diam.; examined for clamp connections but none found.

Gregarious to scattered in humus under oaks in open oak woods. Open oak woods near clay pits, Wellborn, Brazos Co., Texas, April 14, 1952, *H. D. Thiers 1831*, TYPE.

This rhodophyllus is characterized by large, warty to nodulose rather than angular spores, a convex pileus and decurrent lamellae. It is difficult to determine its relationship with other members of the genus. In Singer's system (1) this species would belong in *Rhodocybe*, and seems to be near *Rhodocybe rosciavellanea* from Florida. However, differences in the color of the pileus and spore size make the two distinct.

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SEXUALITY IN SYNCHYTRIUM BROWNII KARLING[1, 2]

B. T. LINGAPPA[3]

(WITH 78 FIGURES)

Sexual reproduction by fusion of isogametes has been reported in a few species of *Synchytrium*, but the nature of sexuality and sex differentiation in the genus has been variously interpreted. Curtis (**1921**) and Köhler (**1930, 1956**) described isogametic fusions in *S. endobioticum*, and Kusano (**1928, 1930**) gave an extensive account and interpretation of their occurrence in *S. fulgens*. Subsequently, Karling (**1954, 1955, 1958**) reported occasional fusion of gametes in *S. brownii*, *S. aureum* and *S. callirrhoeae*. Kusano (**1930**) as well as Köhler (**1930, 1956**) reported that the motile isogametes undergo progressive changes in sex as they age, and in *S. fulgens* Kusano interpreted the subsequent behaviour of the gametes as evidence of relative sexuality. According to his description, as the planospores or prospective gametes emerge from the gametangium they are very actively motile and sexually neutral. Shortly thereafter, their male potency begins to develop and gradually reaches a climax. As they approach the sedentary phase, their maleness becomes weaker and the female potential begins to unfold. By the time they become sedentary their maleness vanishes altogether and the female potential reaches a climax. At this stage, they exert a strong stimulus and attract actively motile males and are most capable of fusion. Unless fusion occurs their femaleness decreases and finally vanishes.

[1] A portion of a Ph.D. thesis submitted to the Graduate School, Purdue University. This study was supported by a grant of the National Science Foundation and a David E. Ross Fellowship. I am highly grateful to my major professor, Dr. J. S. Karling, for guidance and encouragement, and to Drs. G. B. Cummins, C. L. Porter, J. R. Singleton and F. K. Sparrow for help in several ways. Thanks are due to my wife, Yamuna, for assistance and encouragement and to Dr. A. S. Sussman for critically checking the manuscript.

[2] A study of the development, cytology and life-history of this fungus and review of literature have convinced me that *S. brownii* is a synonym of *S. fulgens* Schroet. However, I hesitate to call it *S. fulgens* until the original type of *S. fulgens* is studied or an authentic type is designated.

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Therefore, according to Kusano (**1930**) sex differentiation in *S. fulgens* is expressed in terms of physiological life stages and not in terms of individuals. As a consequence, a gamete (A) may function as a male relative to a sedentary female (B), and later as it ages and becomes sedentary, it functions as a female relative to a motile male gamete (C). Consequently, at any one time, strongly or weakly male or female gametes exist and they exhibit relative sexuality. Thus, Kusano maintained that *S. fulgens* is "monoecious" and its gametes exhibit relative sexuality by the gradual alteration of their sex, and that its behavior differs in several respects from that shown by the gametes of *Ectocarpus* and *Dasycladus* as described and interpreted by Hartman (**1925**) and Jollos (**1926**) respectively.

Köhler (**1930**) extended Kusano's interpretations to the behaviour of the gametes of *S. endobioticum*, and recognized three successive phases. The initial, or Phase I, is characterized by a vigorous motility of the gametes in all directions; Phase II is distinguished by a questing or searching motility (*Suchbewegung*) in which the isogametes seek one another; and Phase III is characterized by the gametes' coming to rest. The gametes in Phase I lack the stimulus to fuse, and fusion occurs only between gametes in Phase II and those in Phase III, the male and female phases respectively. Thus, the gametes pass successively from Phase I to Phase II and Phase III. As soon as a gamete enters Phase III and becomes sedentary it attracts and becomes surrounded by many male gametes of Phase II. As a result numerous clusters of gametes occur; this, according to Köhler, is due to the release of a strong stimulus by the female gametes attracting the males. As soon as the female has fused with one of the males, the supernumerary ones swim away, indicating apparently that the stimulus is no longer released into the surrounding medium. Later Köhler (**1956**) extended this interpretation to *S. fulgens* also on the basis of Kusano's (**1930**) account of sexuality in that species. For several reasons, discussed by Kusano (**1930**), Köhler considered that the gametes of *S. fulgens* do not really show relative sexuality.

Moewus (**1943**), on the other hand, assumed that both male and female gametes are present from the start of motility in *Synchytrium* (which he had not studied) on the basis of his observations on *Chlamydomonas paupera*. In *Synchytrium*, as in *C. paupera*, according to Moewus, the females lose their motility and are brought to rest by a substance secreted by the males. As the first female comes to rest it is surrounded by both males and females, and in such groups other females gradually come to rest under the influence of the males, leading

to the formation of large clusters. Accordingly, Moewus suggested that the quiescent and nonmotile gametes of *Synchytrium* are not former males which have changed to females as Kusano and Köhler reported them to be.

Wolf (**1954**) proposed that the gametes of *Synchytrium* are sexually totipotent, and therefore bisexual or hermophroditic. The manifestation of sexuality in the planospores is related to the imbalance of the sex factors. If these are in balance the planospores are sexually neutral, unable to fuse, and function as zoospores. On the other hand, if an imbalance exists fusion might occur. Wolf postulated that such an imbalance may exist when the gametes are first formed, or it may arise later in response to some external stimuli.

Mrs. Panca Heim (**1956**) recently reported that the motile cells of *S. endobioticum* are like the myxamoebae of the Plasmodiophorales and lack the typical chytrid flagella. Furthermore, she has refuted the reports of fusion of gametes and immediate karyogamy in that species. According to her, the secondary nuclei in multinucleate resting or summer spores (*sporocysts*) fuse in pairs, and meiosis occurs immediately during the last two divisions preceding sporogenesis.

As noted previously, occasional fusion of isogametes was reported by Karling in *S. brownii*, and inasmuch as this species is very similar to or possibly identical with *S. fulgens*, a study of its sexuality has been made for comparison with that of *S. endobioticum* and *S. fulgens* as reported by Curtis, Köhler and Kusano. Ripe sporangia derived from the evanescent prosori as well as from the germinated resting spores were used as sources of planospores and gametes. Single as well as several sporangia were placed in charcoal-treated water, and the behaviour of the planospores was watched under the ordinary as well as the phase-contrast microscope. For immediate examination of the flagella, a drop of Lugol's iodine was added to the suspension of planospores, gametes and zygotes, and the slide was examined under the oil immersion lens. Permanent prepartions of the planospores, gametes and zygotes were made on slides smeared with Haupt's adhesive, fixed in osmium tetroxide fumes, and processed according to the method of Van Den Ende (**1951**). Staining with 1% crystal violet and differentiating with orange G in clove oil also served for the study of their nuclei.

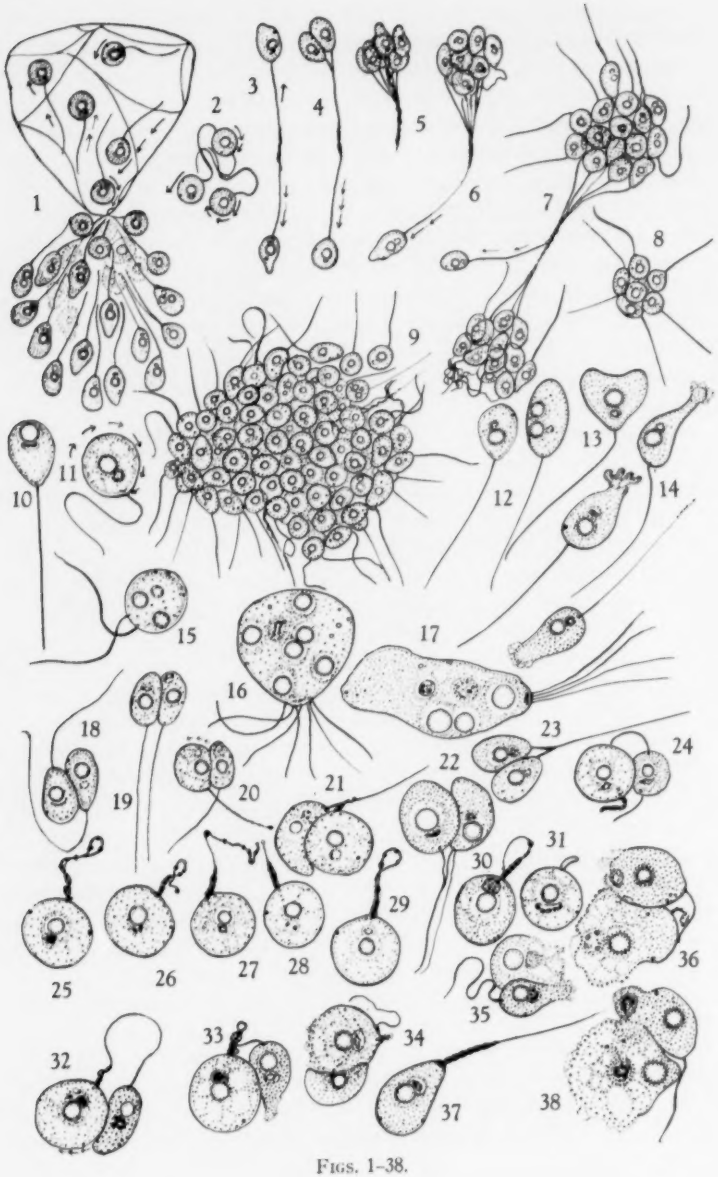
SPOROGENESIS AND PLANOSPORES

The sporangia derived from the evanescent prosori and the resting spores are essentially alike. When placed in fresh water, the ripe sporangia produce planospores at 3° to 30° C. In preparation for sporo-

genesis the homogeneously dispersed refringent orange-yellow globules within the cytoplasm of the sporangia begin to aggregate and to coalesce to form larger globules, one of which will be included ultimately in each planospore. Then, by the cleavage of the cytoplasm, rudiments of planospores are formed, and the mature planospores appear to be suspended in a clear fluid. Concomitant with these changes, one or more hyaline papillae are formed on the sporangia but usually only one of them deliquesces to form an exit pore (FIG. 1). Then the sporangial wall collapses somewhat, and initially a dozen or more planospores emerge passively through the exit. They remain motionless for a few seconds and then swim away. Meanwhile the remaining planospores develop swirling movements within the sporangium, and escape one by one and swim away (FIG. 1). The whole process of sporogenesis takes place in fresh charcoal-treated water within 30 minutes at 20–25°, but it may take 10 hours or more at 10°.

The planospores are oval, oblong, or subspherical and measure 2.5–5 × 3.5–7 μ. Each has a single flagellum 10–16 μ (av. 14 μ) long, an orange-yellow refractive globule and a faintly visible globular nucleus (FIGS. 1–12). When viewed under phase-contrast microscope the nuclei appear to include nodular rods (FIGS. 10–38). In fixed and stained preparations a few nodules are visible on the inside of the nuclear membrane (FIGS. 66–78). Some nuclei appear to contain short and deep-staining, crooked nodular rods, or merely 1–5 globules (FIGS. 66–78). Giant planospores which are 9–16 × 5–14 μ in size, with 2–9 normal flagella and many refractive globules, frequently occur (FIGS. 15–17, 68–70). Similar giant planospores have been described in *S. fulgens* by Kusano (**1930**) and elsewhere in chytrids by Ojerholm (**1934**). They probably arise from unequal cleavage during sporogenesis. As reported by Kusano (**1930**) in *S. fulgens*, they are produced more frequently at 24–27°. At these temperatures sporogenesis occurs very rapidly, and this probably influences the frequency of abnormalities. Curtis (**1921**) suggested that the earlier reports of biflagellate and biguttulate planospores in other species of *Synchytrium* indicated the fusion of gametes. This view, however, is not tenable in *S. brownii* because such planospores are monstrosities.

The planospores are able to remain active just above the melting point of ice but they are killed by freezing. They remain active for over 72 hours at 3° and 2–5 hours at 24°–28°. After this, they become quiescent and settle down, gradually take up water and finally disintegrate unless they find a suitable host. The sedentary planospores are spherical and measure 4–5 μ in diameter and swell up to 15 μ in diameter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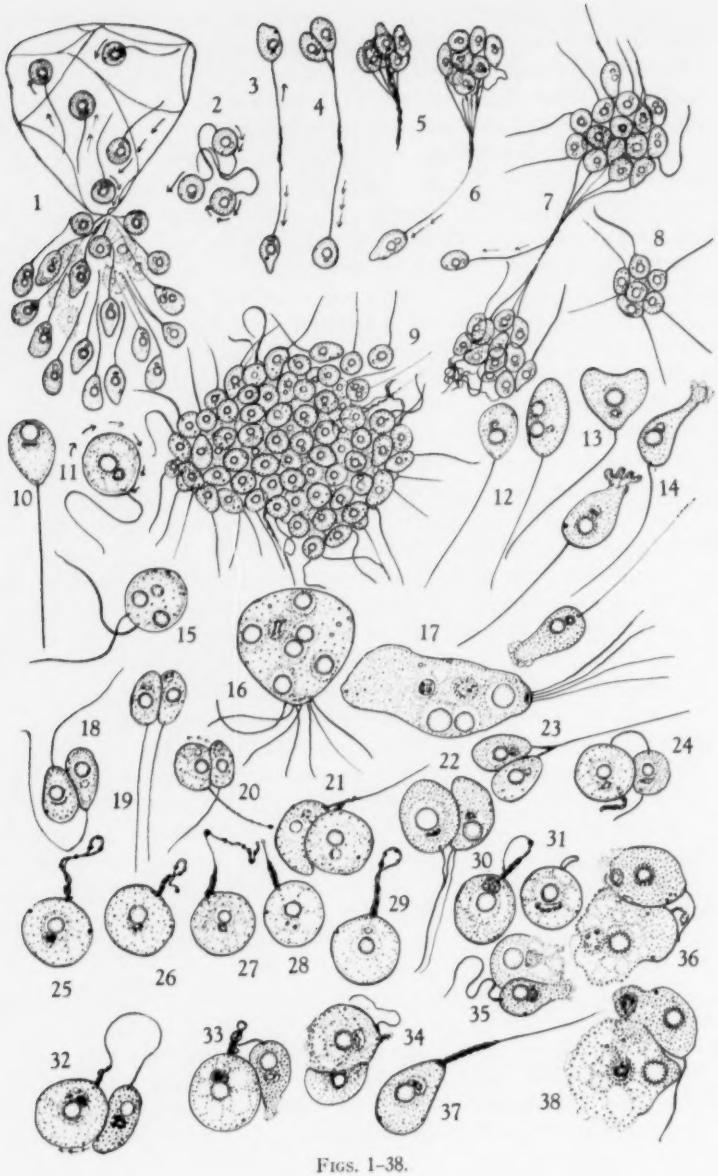
FIGS. 1–38.

as they take up water. As this occurs vacuoles appear in the cytoplasm and discrete granules, in constant Brownian movement, signal the onset of disintegration.

DIFFERENTIATION OF PLANOSPORES INTO GAMETES

Soon after emergence from the sporangia, the planospores swim about actively and aimlessly. After a while, at 20–24°, some of them begin to follow one another, and form actively swimming pairs or groups of three or more (FIGS. 2–9). Also, within a short time after emergence, at temperatures between 5° and 27°, they clump together in large numbers (FIGS. 5–9) in the vicinity in the empty sporangia. The pairs and clumps are formed regardless of the temperature at which the sporangia are ripened, and in all preparations some planospores do not form pairs or aggregates but continue to swim about singly. These settle down ultimately and become spherical or subspherical but retain their flagella (FIG. 10). They either disintegrate or attach themselves to the host

FIGS. 1–38. Planospores, gametes and copulation in *Synchytrium brownii*, drawn from living preparations. FIG. 1. Emergence of planospores, × 750. FIG. 2. Closely associated planospores exhibiting intermittent circular movements, × 800. FIG. 3. Gametes attached by their flagella, × 800. FIG. 4. Planospore attached to a pair and dragging it in opposite direction, × 840. FIG. 5. Cluster of planospores with entangled flagella, × 800. FIG. 6. Planospore struggling to free from the cluster, × 800. FIG. 7. Clusters entangled by flagella, × 800. FIG. 8. Cluster of planospores; their flagella are free, × 800. FIG. 9. A large clump of planospores, × 800. FIG. 10. Sedentary planospore, × 1500. FIG. 11. Planospore spinning intermittently, × 1500. FIG. 12. Normal active planospores with one or two oil globules, × 1500. FIG. 13. Sedentary planospore, × 1600. FIG. 14. Sedentary amoeboid planospores, × 1600. FIG. 15. Planospore with 2 flagella and 2 oil globules, × 1500. FIGS. 16, 17. Multiflagellate giant planospores, × 1500. FIG. 18. Intermittently quiescent pair of gametes, × 1200. FIG. 19. Actively swimming pair of gametes, × 1200. FIG. 20. Flagellate active gamete pushing against a sluggish (comparatively larger) gamete, × 1000. FIG. 21. Sedentary pair of gametes; flagellum of spherical gamete much shortened, × 1200. FIG. 22. Sedentary pair of gametes; one with two flagella, × 1500. FIG. 23. Sedentary pair, the two flagella fused, × 1200. FIG. 24. Sedentary pair, the larger more hydrated and with a very short and stout flagellum (due to gradual absorption), × 1500. FIGS. 25–30. Stages in the absorption of flagella of the sedentary member of stable pairs of gametes, × 2000. FIG. 31. Remnant of the absorbed flagellum, × 2000. FIG. 32. Active gamete pushing or crawling over the distended sedentary gamete, × 2000. FIG. 33. Sedentary pair, anterior end of the smaller gamete pseudopodium-like, × 2000. FIG. 34. Anterior end of the distended gamete pseudopodium-like, × 2000. FIGS. 35, 36. Anterior ends of both gametes spreading and retracting like pseudopodia, × 2000. FIG. 37. Sedentary planospore, base of its flagellum much thickened, × 1600. FIG. 38. Fusion of gametes, × 2000.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FIGS. 1–38.

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DIFFERENTIATION OF PLANOSPORES INTO GAMETES

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surface, absorb their flagella and infect the host cells. Therefore, they function as zoospores.

In actively swimming pairs both gametes are morphologically identical in the beginning. They may continue to swim together or suddenly separate. If the pairs remain together and fuse, they are referred to as stable pairs. In such pairs, one of the gametes gradually begins to absorb its flagellum and becomes sedentary earlier than the other gamete (FIGS. 32–36, 75, 76). Also, among the free-swimming planospores some become sluggish as their flagella are gradually absorbed, and are surrounded by active gametes. Thus gametes of two mating types are distinguishable, namely a sedentary receptive type (FIGS. 25–31) and an active searching type (FIGS. 32–36). The gametes of the latter type retain their flagella after becoming sedentary whereas those of the former type absorb their flagella gradually and become sedentary earlier. The gametes may pair either before or after such differentiation (FIGS. 18–24). In any event, all stable pairs consist of flagellate and aflagellate gametes at the time of fusion (FIGS. 35, 38).

Within 10 minutes after their emergence, as few as 1 in 300 of the planospores may have partially absorbed the flagella, and 3 hours later about 20% of them may be aflagellate. This was repeatedly determined by adding to the suspension a drop of Lugol's iodine, which kills the planospores and clearly reveals the presence of flagella. Six hours after emergence the planospores cease their activity. Among these can be found zygotes and partially flagellate, aflagellate and numerous flagellate individuals (FIGS. 9, 67). This indicates that not all planospores undergo the same degree of differentiation and that not all sedentary ones become aflagellate. This conclusion has been reached after following the activities of single planospores and mixtures of planospores in individual droplets of water from the time of emergence to ultimate quiescence.

CLUMPS

As noted previously, large and small clumps of planospores occur in *S. brownii* (FIGS. 5–9, 67, 73, 74). Such clumps have been described also by Kusano (**1930**) and Köhler (**1930**, **1956**) for *S. fulgens* and *S. endobioticum* respectively. These clumps are formed in light as well as in darkness, at 5–27°. They are irregularly distributed in suspensions of planospores whether derived from a single sporangium or from numerous sporangia. The aggregation of planospores, therefore, is not due to phototaxis as Kusano (**1930**) reported for *S. fulgens*. Köhler (**1932**) reported that aggregation of gametes could be induced by the

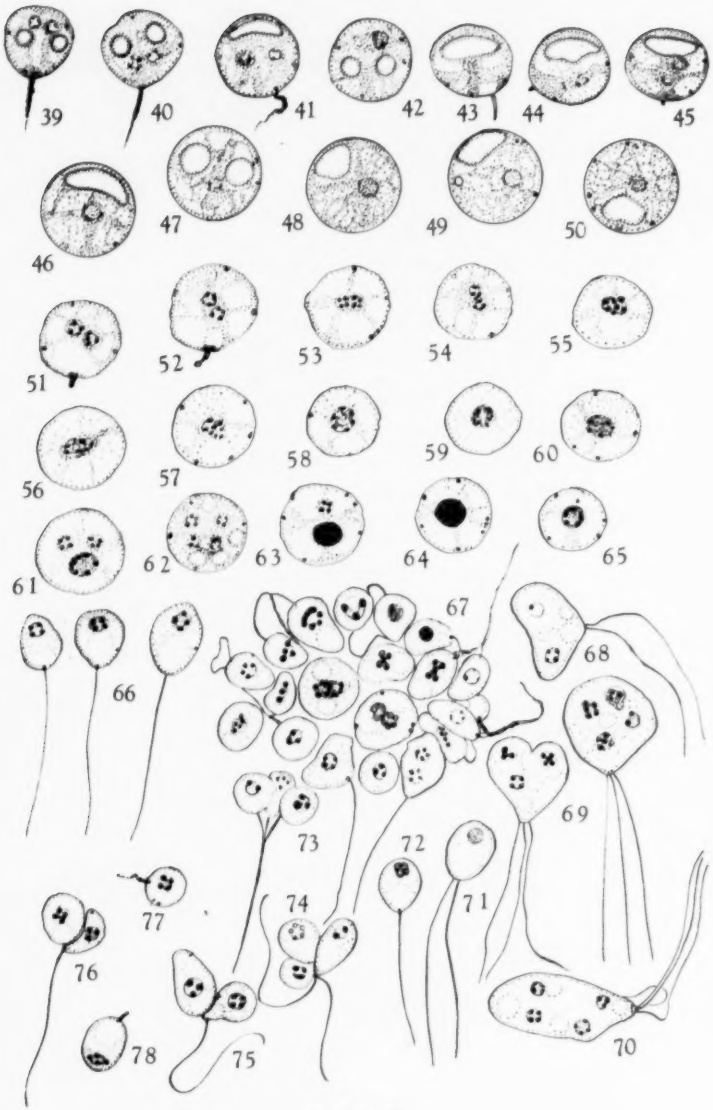
addition of droplets of vaseline to the suspension of planospores in *S. endobioticum*. He explained this by assuming that vaseline enhanced the alteration and evolution of sex in the planospores. However, in *S. brownii* aggregates were always formed in fresh water suspensions of planospores and the addition of vaseline droplets neither increased nor decreased the number and size of the aggregates nor did it induce the clumping of the remaining free swimming planospores. In *S. brownii* clumps are formed quickly after the planospores emerge, and after about 30 minutes the number or size of the aggregates does not increase in any suspension.

The clumps of planospores do not emerge as such from the sporangia but are formed by the aggregation of free-swimming planospores in the vicinity of the empty sporangia. They appear to form by collision and aggregation of active planospores whose flagella become entangled (FIGS. 3–7). Small clusters and separate planospores are often stuck to a clump and actively struggle to pull loose from it. The flagella which are in the process of absorption appear to be very sticky, and they probably initiate clumping and cluster formation. However, in any suspension, not all the free-swimming planospores aggregate to form a single or a few very large clumps.

Individual planospores in a clump continue to push against one another and ultimately become sedentary. One to two hours after the individual planospores in the clumps have become sedentary, stained preparations show large numbers of flagellate individuals along with a few aflagellate ones (FIG. 67). There may be 3–4 zygotes or none at all in a clump of more than one hundred planospores. On the other hand, elsewhere in the same preparation large numbers of separate zygotes may be present.

ABSORPTION OF FLAGELLA

As one of the gametes of a stable pair becomes sluggish its flagellum becomes swollen terminally, and becomes crooked, beaded or looped (FIGS. 24–30). By the time the sluggish gamete has become sedentary its flagellum remains as a short stump at the proximal end (FIGS. 31, 34, 36, 77, 78). The same process of gradual absorption of flagella occurs in the zoospores if they settle on the host surface. Repeated observations so far indicate that gradual absorption of flagellum occurs in only one of the gametes of a pair while the flagellum of the other gamete is progressively absorbed only after plasmogamy has occurred. However, the flagella of zoospores are absorbed only if they settle on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FIGS. 39–78.

the host surface, whereas those that do not do so disintegrate along with the flagella after excessive hydration.

In a suspension of copulating gametes unstable pairing occurs—i.e., a pair may remain together for a few minutes to two hours or more but suddenly separate and swim away. One of the gametes of such a pair may be less active and have a partially absorbed flagellum. Active gametes follow and pair with such sluggish gametes. The partially absorbed and beaded flagella appear to be sticky because normal flagella of other planospores or gametes get stuck to them so firmly that individuals so entangled strain in opposite directions to free themselves without any success (FIGS. 3–7). At later stages of absorption, the stumps appear empty and finally disappear. The flagella of the zygotes are also absorbed in the same manner (FIGS. 39–41, 43, 51, 52).

COPULATION

As described above, the gametes are differentiated into sedentary and active types. When active isogametes pair differentiation apparently occurs during the close of their motility. Initially such pairs swim together for two hours or more at 24°, and then stop intermittently and swim again. The frequency and duration of stoppage or inactivity increases with time, and one of the gametes of the stable pairs becomes increasingly sluggish and ultimately sedentary. The other gamete, however, continues to jerk about for a longer time (FIG. 20). One or more active gametes may approach active or temporarily quiescent pairs. Such stray gametes swim away unless entangled by their flagella. Many pairs of gametes swim together for a long time, intermittently stop and

FIGS. 39–78. Zygotes, planospores and gametes of *Synchytrium brownii*. FIGS. 39–50. Zygotes drawn from living preparations, × 2000. FIGS. 51–78. Drawn from fixed and stained preparations; FIGS. 51–65, × 2000. FIGS. 66–78, × 1500. FIGS. 39, 40. Zygotes shortly after plasmogamy. FIG. 41. Zygote with fused oil globules. FIG. 42. Zygote after karyogamy. FIGS. 43–45. Stage of absorption of flagellum. FIGS. 46–50. Zygotes, showing characteristic appearance of cytoplasm. FIGS. 51–60. Showing karyogamy. FIG. 61. Zygote containing a diploid and two haploid supernumerary nuclei. FIG. 62. Zygote containing four haploid nuclei. FIG. 63. Zygote containing diploid and a haploid nuclei. FIGS. 64, 65. Zygotes containing diploid nuclei. FIGS. 66–78. Planospores and gametes. Note the appearance of their nuclei in stained preparations. FIG. 66. Planospores soon after emergence. FIG. 67. Clump, showing two zygotes amidst flagellate and aflagellate gametes; fixed 10 hours after zoosporogensis. FIGS. 68–70. Giant planospores. FIG. 71. Planospore—biflagellate but uninucleate. FIG. 72. Planospore fixed two hours after becoming sedentary. FIGS. 73, 74. Small clusters of planospores. FIGS. 75, 76. Pairs of gametes. FIGS. 77, 78. Sedentary gametes with partially absorbed flagella.

swim and ultimately separate suddenly, and they may or may not pair with other gametes. Various temporary and unstable combinations of this nature occur, and in this respect observations on the activity of gametes of *S. brownii* agree with those of Kusano (**1930**) for *S. fulgens*.

In stable pairs, the active gametes continue jerking movements, pushing against and crawling over the sedentary gametes (FIGS. 20, 21, 32). When a pair remains together in this condition for about an hour the sedentary gamete becomes hydrated and aflagellate. Its body is subspherical and much larger than that of its mate which is oval or elongate and has denser content and an intact flagellum (FIGS. 34, 35). The latter has also ceased its movements by this time. In such pairs, a portion of the much hydrated gamete begins to develop pseudopodium-like protrusions (FIG. 34), and shortly afterward the anterior end of the other gamete also develops similar protrusions (FIGS. 35, 36). The anterior ends of both gametes gradually and intermittently spread out and retract; and in this process their pseudopodium-like ends come into close contact (FIGS. 35, 36). At some stage in this process their plasma boundaries fuse, and the contents intermingle (FIGS. 36, 38). Within 1–3 minutes the two gametes fuse and plasmogamy is thus completed. At the time of fusion both gametes are sedentary, but the one that became sedentary first is aflagellate and hydrated. The other gamete has retained its flagellum so that the zygote is uniflagellate. However, the zygote does not become motile or amoeboid; and it contains two nuclei and two refractive globules.

Within 30 minutes after plasmogamy the remaining flagellum is gradually absorbed and the two nuclei fuse (FIGS. 51–60). The refractive globules also fuse into an irregular mass which remains near the periphery. At this time, the zygote is spherical, 4–6 μ in diameter, with a semirigid membrane. The zygotes are characteristically vacuolate (FIGS. 39–65). They appear similar to the zygotes of *S. fulgens* and *S. endobioticum* as figured by Kusano (**1930**), Curtis (**1921**) and Köhler (**1932**), respectively.

Before karyogamy, the gametic nuclei include nodular, crooked, short and deeply stained rods or 1–5 globules (FIGS. 51–63). Soon after karyogamy the diploid nucleus contains a tangled mass of deep-staining rods (FIGS. 53–60, 65), but shortly thereafter it stains deeply throughout (FIG. 63, 64). The gametic nuclei as well as the zoospore nuclei measure about 1.5 μ in diameter, whereas the diploid nuclei measure 2 to 2.5 μ. Occasionally a zygote may contain more than two haploid nuclei, or one or two haploid nuclei in addition to a diploid one (FIGS. 61–63). Such zygotes might have resulted from the fusion of more than two gametes, or one or both gametes of the pair might have been

abnormal and contained more than one nucleus. Occurrence of multiple fusion of gametes is known in *S. fulgens* and in *S. endobioticum* according to Kusano (**1930**) and Köhler (**1932**) respectively. In *S. endobioticum* the supernumerary nuclei are known to degenerate, but their fate is not known in *S. brownii*.

DISCUSSION

The planospores of *S. brownii* may function as zoospores and reinfect the host or differentiate into two types of gametes and fuse. No evidence of a change from male to female in the gametes has been observed. However, as described by Kusano (**1930**) for *S. fulgens*, the planospores of *S. brownii* also form pairs, clusters, and large clumps. In such aggregations the flagella of the planospores are entangled, and this suggests that the entanglement may be a factor in clumping. However, the flagella of several spores in the aggregates may be free, and small clusters have been observed without any entangled flagella. Such occurrences suggest the operation of some kind of chemotactic or gamone mechanism. In algae which produce iso- or anisomorphic zoogametes and exhibit relative sexuality, similar clumps, clusters or pairs occur commonly. The size of the aggregates has been taken as a measure of the relative sex valence of the gametes. Of several such aggregations referred to by Hartman (**1956**) those of *Ectocarpus siliculosus* and *Dasycladus clavaeformis* are essentially similar to those of *S. Brownii*. Previously Kusano's (**1930**) interpretation of relative sexuality in *S. fulgens* was based mainly on its similarity to the sexuality exhibited by these algae. However, no evidence of such sexuality has been found in *S. brownii*. In *S. brownii* aggregation or clumping does not appear to be an expression of sexuality, because only a few, if any, zygotes are found in a clump of hundred or more planospores. In *Chlamydomonas cugamatos* also, Hartman (**1956**) has pointed out that although different types of aggregations may occur conspicuously, they do not involve relative sexuality. The behaviour of the planospores after their emergence indicates that some of them become differentiated into sexually opposite types of gametes—flagellate and aflagellate—at the time of fusion. Thus far, copulation between two gametes of the same type—fusion between two flagellate or two aflagellate gametes—has not been observed. Of Hartman's (**1956**) criteria of sexuality, bisexual potency, bipolar bisexuality and relative sexuality, only the bisexual potency of the planospores can be inferred for *S. brownii*. For several reasons, Kusano (**1930**) himself was critical of the occurrence of relative sexuality in *S. fulgens*, and Köhler (**1956**) also questioned its occurrence in *S. fulgens*.

In *S. brownii* sexuality is expressed irrespective of whether the gametes are derived from the same sporangium or from different sporangia derived from the evanescent prosori or germinated resting spores. Also, over-ripening of the sporangia does not influence the expression of sexuality of the gametes emerging from them. Kusano (**1930**) arrived at the same conclusions for *S. fulgens*. However, Curtis (**1921**) and Köhler (**1956**) reported that in *S. endobioticum* over-ripening of the sporangia leads to differentiation of planospores into gametes capable of fusion. According to Curtis (**1921**) gametes become differentiated, fuse and give rise to zygotes even within over-ripened sporangia. In *S. brownii*, irrespective of how long the sporangia are retained after ripening, their contents do not cleave into individual spores unless fresh water is provided.

SUMMARY

Planospores of *S. brownii* are sexually neutral in the beginning. Some of them gradually develop sexual tendencies and differentiate into two types of gametes, a receptive type which is the first to become sedentary and to absorb its flagellum, and a searching flagellate type which remains active for a longer time and retains its flagellum after becoming quiescent. Copulation occurs between gametes of these two types. As far as is known, neither alteration of sex of the gametes nor relative sexuality occurs.

About 1–3 hours after pairing plasmogany occurs and within half an hour it is followed by karyogamy. The zygote is nonmotile, though it has a flagellum for a brief period. The oil globules also fuse in the zygotes. Occasionally the zygotes may have one or more supernumerary nuclei.

Along with normal planospores, giant planospores are frequently produced which possess several normal flagella, nuclei and oil globules. They are considered to be monstrosities arising from unequal cleavage. The planospores that do not differentiate into gametes function as zoospores, infect the host, and develop into evanescent prosori. The flagella of the zoospores, gametes and zygotes are gradually absorbed and are not dropped off. Planospores emerging from sporangia of evanescent prosori or from germinated resting spores behave similarly. Also, differentiation of planospores into gametes is not hastened by over-ripening of the sporangia.

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THE GROWTH-PROMOTING EFFECT OF SEVERAL AMINO ACIDS ON THE COMMON CULTIVATED MUSHROOM, AGARICUS BISPORUS [1]

IAN M. FRASER AND BYRON S. FUJIKAWA

Singer (**14**) has pointed out that the correct designation for the common cultivated mushroom is *Agaricus bisporus* (Lange) Singer. Lange (**10**) described two forms, viz. the white-capped (forma *albida*) and the brown-capped (forma *avellanea*).

The most extensive investigation of the nutrient requirements of this organism on a synthetic medium is that of the Danish investigator Treschow (**16**), using principally the brown-capped form. With a basal medium of glucose, asparagine and mineral salts, he found that addition of thiamine or biotin allowed good growth of unshaken floating cultures. However, considerably better growth occurred when extracts of yeast or alfalfa or nettle leaves were added.

Humfeld and Sugihara (**8**) studied the nutrient requirements of specially selected sterile isolates of the cultivated mushroom, designated by them as *Agaricus campestris*. They found that good growth occurred in shaken submerged cultures in a synthetic medium containing glucose, urea and mineral salts without addition of any vitamins or other growth factors. However, the majority of the strains they isolated grew very poorly under these conditions.

In previous studies on an Australian strain of the white-capped form (**6**) using techniques similar to those of Treschow (**16**), it was found that only a small amount of growth occurred in the presence of thiamine or a mixture of eight B vitamins but that the addition of yeast extract or mushroom-compost extract led to vigorous growth. Indole-3-acetic acid was also found to promote growth but the amount of this substance present in the extracts, as determined by the *Avena*-coleoptile-curvature assay, was not adequate to account for their growth-promoting activity

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(**6**). The studies reported here arose from an interest in determining the nature of the growth promoting factor(s) in these extracts.

These investigations have centered on the possibility that the presence of one or more specific amino acids might be responsible for the growth-promoting effect. Treschow (**16**) found that dl-asparagine was the best single nitrogen source of those tested, although dl-glutamic acid was only slightly inferior. Glycine, d-alpha-alanine, l-leucine and simple ammonium salts were less satisfactory, the growth in their presence decreasing in the order they are listed. In testing these nitrogen sources, Treschow (**16**) used a synthetic medium supplemented with yeast extract, a source of vitamins and amino acids. However, with asparagine as the nitrogen source, thiamine or biotin was capable of partially substituting for the yeast extract. Asparagine alone is a good source of nitrogen for most Basidiomycetes (**9**) but it seemed possible that our Australian strain and possibly others might not be able to synthesize all their amino acid requirements from this substance.

MATERIALS AND METHODS

Three strains of *Agaricus bisporus* were employed in this study. One, designated as PS1, is a substrain of the Australian isolate previously employed (**6**). PS2 is another substrain of this isolate characterized by a more rapid growth rate than PS1 as well as some nutritional differences to be described in this paper. These two substrains were maintained independently under different conditions in two widely separated laboratories for a period of several years. PS5 is an American isolate obtained from the center of a young cultivated mushroom cap supplied by Mr. Frank Walton, Gardena, California. All these isolates came from fruit bodies conforming closely to the description of the white-capped form given by Lange (**10**). Stock cultures of all strains were maintained on slants of "Bacto" malt agar containing 5 g/l "Bacto" yeast extract.

The basal synthetic medium was similar to that used previously (**6**) and was a slight modification of the medium developed by Treschow (**16**) for optimal growth of the brown-capped form of *A. bisporus*. The amounts of the various constituents per liter were as follows: glucose, 10 g; l-asparagine, 1 g; potassium chloride, 0.2 g; magnesium sulfate ($\cdot 7H_2O$), 0.2 g; potassium dihydrogen phosphate, 0.14 g; sodium monohydrogen phosphate ($\cdot 12H_2O$), 1.9 g; calcium chloride ($\cdot 2H_2O$), 0.27 g; ferric chloride ($\cdot 6H_2O$), 0.017 g; boron (as boric acid), 0.01 mg; copper (as copper sulfate), 0.1 mg; manganese (as manganese sulfate), 0.02 mg; molybdenum (as ammonium molybdate),

0.02 mg; zinc (as zinc sulfate), 2.0 mg; distilled water to make one liter. Observations made during its use in the initial experiments of this investigation led to its further modification to contain 30 or 40 g/l of glucose and 3 or 4 g/l of asparagine as described in more detail under Results.

The concentrations per liter of the vitamins and amino acids employed in various combinations in the different experiments are as follows: thiamine hydrochloride, 0.02 mg; biotin, 0.001 mg; inositol, 20 mg; nicotinamide, 0.024 mg; calcium pantothenate, 0.044 mg; pyridoxal hydrochloride, 0.041 mg; p-aminobenzoic acid, 0.027 mg; riboflavin, 0.075 mg; folic acid, 0.02 mg; vitamin B_{12}, 0.004 mg; l-glutamic acid, 552 mg; l-leucine, 384 mg; l-proline, 208 mg; dl-lysine, 296 mg; l-tyrosine, 128 mg; dl-valine, 256 mg; dl-isoleucine, 256 mg; dl-threonine, 232 mg; dl-aspartic acid, 208 mg; dl-phenylalanine, 192 mg; l-arginine, 96 mg; dl-methionine, 144 mg; l-histidine, 48 mg; dl-alanine, 96 mg; l-tryptophane, 32 mg; glycine, 12.8 mg; dl-serine, 20.8 mg; l-cystine, 8.0 mg; l-hydroxyproline, 4.8 mg. The first seven vitamins listed were present in the 7-B-vitamin mixture referred to subsequently. Because of their frequent use in the tables, five amino acids are referred to by the following standard abbreviations (**4**): dl-methionine, Met; dl-phenylalanine, Phe; l-proline, Pro; l-tryptophane, Try; l-tyrosine, Tyr. The concentrations of the amino acids were selected to supply amounts equivalent to those present in 2.5 g/l acid-hydrolysed casein according to the manufacturer (Nutritional Biochemicals Corporation), with the exception of l-tryptophane which was destroyed at least in part by hydrolysis. Where only dl-amino acids were available, double quantities were employed (**9**). The glucose and inorganic chemicals used were "Baker Analyzed" reagents. The l-asparagine, amino acids, most of the vitamins and the "vitamin free" casein-hydrolysate-acid were obtained from Nutritional Biochemicals Corporation. The thiamine and folic acid were donated by Merck, Inc. The yeast extract was obtained from Difco Laboratories, Inc.

The medium was usually prepared in double strength and then diluted by the addition of vitamin or amino-acid solutions or water to the appropriate volume. After autoclaving for ten minutes at 15 lb steam pressure the medium had a pH of 7.0.

Inoculum for the experiments consisted of 2 mm discs cut at from 0.5–1.5 cm behind the growing edge of 4–8-week-old cultures in Petri dishes. The basal synthetic medium (15 ml per plate) without further addition except for 20 g/l "Bacto" agar for solidification allowed sufficient growth of PS2 but for PS1 the presence of 2.5 g/l yeast extract was

also necessary. Initially PS5 was grown on the same medium used for PS1 but preliminary experiments showed that better growth was made on the basal medium modified to contain 30 g/l of glucose and 3 g/l of asparagine, together with half the usual quantities of the 7 B vitamins and the amino acids phenylalanine, methionine, proline and tryptophane.

The organisms were grown as floating cultures on 25 ml of the liquid medium in 125 ml "Pyrex" Erlenmeyer flasks. The discs of inoculum were carefully floated on the surface. In initial exploratory experiments a growth period of 14 days was employed but, as previously (**6**), a 28 day growth period was used for most of the final data presented. The cultures were incubated at 25° C. At the completion of the experiment the mycelium was filtered off, dried at 100° for 18 hours and weighed.

In every experiment five replicate cultures were set up for each treatment and the mean dry weight calculated. Contamination by other organisms occasionally reduced the final number to four or, rarely, three. Most of the experiments were repeated once or more, at least in their key parts, and the mean dry weight of all of them for each treatment is shown in the tables. Where parallel experiments under slightly different conditions led to the same conclusion, precise repetition of the experiments was not deemed necessary in most cases.

The considerable variation in growth response encountered between replicate cultures and experiments made statistical testing of differences between means desirable. Analysis of variance was performed according to the methods of Snedecor (**15**). The difference between means (D) necessary for significance at the 0.05 probability level was also calculated (**15**) and is appended to the tables of results. The t-test has also been employed in some instances to determine the significance of differences between two means without regard to the variation encountered in the rest of the experiment.

RESULTS

In initial efforts to duplicate the previously observed growth-promoting effect of yeast and compost extracts on the Australian isolates (**6**), two additional B vitamins, folic acid and vitamin B_{12}, were tried in combination with the 8 B vitamins previously tested. They produced no growth response nor did the addition of hemin (20 mg/l), a growth factor for another coprophilous fungus, *Pilobolus kleinii* (**13**).

The effects of additional sources of nitrogen in the form of an amino-acid mixture were examined. Acid-hydrolysed casein (2.5 g/l) was

found to produce a growth response with all three isolates approximately equal to that obtained with "Difco" yeast extract (5 gm/l). A synthetic mixture of amino acids simulating casein hydrolysate was equally active.

In an endeavor to determine which one or more of the nineteen amino acids present in casein hydrolysate must be responsible for the observed growth promoting activity, each one was tested singly under the same conditions in amounts equivalent to those occurring in casein. In the case of the American isolate PS5 and the Australian isolate PS2, each of the amino acids phenylalanine, methionine, proline, tryptophane and tyrosine, when supplied alone, was found to promote growth to some degree. The response to phenylalanine and methionine was greater than to the other three. With the Australian isolate PS1, proline was the only amino acid which, when added alone, produced any growth response. The response to proline was small but the addition along with it of phenylalanine, methionine, tryptophane or tyrosine singly or in combination produced a greater response, phenylalanine being particularly effective. With the other two isolates numerous combinations of the five amino acids also produced a greater growth response than the single amino acids.

Since the addition of these amino acids significantly enhanced the nitrogen and carbon content of the medium and since the glucose and asparagine levels of Treschow's (**16**) medium as originally employed were rather low in comparison to many fungal media, it seemed of interest to investigate the effect of progressively increasing these while holding the inorganic constituents, vitamins and amino acids at the same level. The tests were conducted with the addition of several single amino acids and several combinations of amino acids. However, in TABLE 1 only the results obtained with a combination of all five amino acids are shown, since this combination produced the greatest growth response.

The data in TABLE 1 were obtained in only a single experiment with each isolate but the results are in qualitative agreement with those with single amino acids and other combinations. Increasing the concentrations of glucose and asparagine from the original level of 10 g/l and 1 g/l respectively produced greater growth. The optimal growth response was produced by glucose concentrations of 30–40 g/l and by asparagine concentrations of 3–4 g/l. Experiments not reported in detail in which the 30 or 40 g/l level of glucose was combined with the original 1 g/l of asparagine or the 10 g/l level of glucose was combined with the 3 or 4 g/l level of asparagine indicated that the increase in glucose was more

important than the increase in asparagine but that the simultaneous increase of both produced the best response. Doubling the concentration of inorganic nutrients and vitamins was without further significant effect (TABLE 1).

The effect of the various amino acids found to promote growth in the preliminary experiments was now re-examined using these more optimal levels of glucose and asparagine. With isolates PS5 and PS2 a glucose level of 40 g/l and an asparagine level of 4 g/l were selected as optimal. With PS1, levels of 30 g/l of glucose and 3 g/l of asparagine were chosen as most nearly optimal since there was some evidence of inhibition by higher levels, as seen in TABLE 1 and in the data obtained, but not given in detail, with other amino-acid combinations.

TABLE 1

THE EFFECT OF DIFFERENT LEVELS OF GLUCOSE AND ASPARAGINE ON THE GROWTH OF SEVERAL STRAINS OF AGARICUS BISPORUS IN THE PRESENCE OF 7 B-VITAMIN MIXTURE AND Phe, Met, Pro, Try AND Tyr

Addition per liter of medium		Dry Weight		
Glucose g	Asparagine g	Strain PS5 mg	Strain PS2 mg	Strain PS1 mg
10	1	75	114	31
20	2	119	189	61
30	3	131	223	78
30*	3*	—	—	70
40	4	143	241	65
40*	4*	112	265	—
60	6	109	210	71
		(D = 54)	(D = 63)	(D = 21)

* Rest of medium at 2× normal strength.

Almost all the possible combinations of the five amino acids were tested with the optimal media but only those producing the most significant results are included in TABLE 2. The data in this table represent, in the majority of cases, the mean of several separate experiments. The most obviously important single amino acids or combinations were tested three or four times while the others shown were tested twice or occasionally only the once. Qualitatively the results in all the experiments were in agreement, although unfortunately there was considerable quantitative variation in different experiments. This is reflected in the large D values.

The results in TABLE 2 show that despite the increased glucose and asparagine levels the presence of certain amino acids was necessary for appreciable growth to take place. With PS5 and PS2, the addition of

either phenylalanine or methionine allowed good growth. A combination of the two was superior to either alone, although statistically so only upon the further addition of proline for PS2 and of a combination of proline, tryptophane and tyrosine for PS5. However, in this optimal medium the three amino acids proline, tryptophane and tyrosine produced statistically insignificant increases in the growth of isolates PS5 and PS2 when added alone or in combination with phenylalanine and methionine. The response of isolate PS5 to casein hydrolysate compared to that of phenylalanine or methionine was much less in this

TABLE 2

THE EFFECT OF VARIOUS AMINO ACIDS AND VITAMINS ON THE GROWTH OF SEVERAL STRAINS OF AGARICUS BISPORUS ON MODIFIED BASAL MEDIUM

Treatment	Dry weight		
	Strain PS5* mg	Strain PS2* mg	Strain PS1** mg
7 B-vitamins	8	25	3
7 B-vitamins + casein hydrolysate	36	164	46
7 B-vitamius + Phe	85	174	—
7 B-vitamins + Met	72	154	—
7 B-vitamins + Pro	10	41	31
7 B-vitamins + Phe + Met	113	202	—
7 B-vitamins + Phe + Pro	70	—	95
7 B-vitamins + Phe + Met + Pro	118	234	77
7 B-vitamins + Phe + Met + Try + Tyr	115	—	12
7 B-vitamins + Phe + Met + Pro + Try + Tyr	132	229	100
Phe + Met + Pro + Try + Tyr	22	—	12
Thiamine + Phe + Met + Pro + Try + Tyr	120 (D = 48)	224 (D = 46)	82 (D = 37)

* Basal medium modified to contain 40 g/l glucose and 4 g/l asparagine.
** Basal medium modified to contain 30 g/l glucose and 3 g/l asparagine.

optimal medium than in the preliminary experiments with the original medium of Treschow (**16**).

In accordance with the results of the preliminary experiments, strain PS1 differed from PS5 and PS2 in amino-acid requirements on the optimal medium. The only single amino acid capable of inducing any growth response was proline. The increase in growth over that with no addition shown in TABLE 2 is not statistically significant when a D value based on the total variation encountered with all treatments is employed in testing but is significant in a t-test employing only the data of these two treatments. Unless proline was present the response to any other amino acid was negligible, as shown, for example, in TABLE 2

by the very small growth produced with the combination of phenylalanine, methionine, tryptophane and tyrosine. Phenylalanine was the only amino acid which, when added along with proline, produced a significant increase in growth. The addition of methionine, tryptophane or tyrosine alone or in combination, along with proline was without significant effect. In this optimal medium the response to casein hydrolysate was less than that to the combination of proline and phenylalanine. In the preliminary experiments this difference was much less marked.

All three strains grew very little in the presence of either the vitamin mixture alone or the five-amino-acid mixture alone (TABLE 2), indicating that the presence of one or more of the vitamins was necessary for significant growth to take place in the presence of the amino-acid mixture. As shown in TABLE 2, the addition of only thiamine along with the five-amino-acid mixture produced a growth response approximately equal to that upon addition of the seven-vitamin mixture. None of the other constituents of the vitamin mixture produced any growth-promoting effect in the presence of the five-amino-acid mixture. Thiamine was thus the only vitamin responsible for the growth response produced by the mixture.

DISCUSSION

The results obtained can be interpreted in terms of some inability of these various strains of *Agaricus bisporus* to synthesize several amino acids and the vitamin thiamine in amounts adequate to reveal the maximum growth potentialities under these experimental conditions. Although variation of the levels of the several amino acids from the more or less arbitrarily employed ones might reveal the exact relationship more clearly, it is believed that some general conclusions can be drawn from the results so far obtained.

All three strains were unable to synthesize significant amounts of thiamine in terms of their needs for growth. The synthetic capacity for the other B vitamins appeared to be adequate. However, in his work with the brown-capped form, Treschow (**16**) found a deficiency in the formation of biotin also. An inability to synthesize thiamine appears to be a common feature amongst the Basidiomycetes (**9**).

Several facts show that the amino acids that promote growth are meeting specific requirements rather than the general nitrogen requirements of the organism. The amounts of these amino acids added are quite inadequate to supply the nitrogen present in the mycelial growth in their presence. Using an average figure for the nitrogen in the mycelium of 5% of the dry weight (**5**), 100 mg dry weight of growth

with dl-phenylalanine (TABLE 2) in 25 ml of medium in a flask would contain 5 mg of nitrogen. The dl-phenylalanine added at 192 mg/l or approximately 5 mg/25 ml could supply at most 0.4 mg of nitrogen per flask or half this if only the l-form were utilized (**9**). Similar results can be obtained for methionine. With proline alone, the growth response of PS1 was much smaller but it still supplied inadequate nitrogen unless the mycelium nitrogen content was considerably lower than 5%. Consequently, the asparagine in the medium must be supplying most of the nitrogen required for the growth of the organism while the phenylalanine, methionine and probably proline supply specific needs occasioned by a reduced ability to synthesize these amino acids from asparagine. This is supported by a preliminary experiment with strain PS2 in which we examined the ability of these amino acids to serve as the sole source of nitrogen when added at such a concentration that they supplied an amount of nitrogen equal to that supplied by 1 gm/l asparagine. Trial of higher concentrations was prevented by solubility limitations with some of them. With phenylalanine as the sole source of nitrogen the growth was the same as with asparagine. With methionine and particularly proline there was a little more growth but still less than that obtained when either of these was combined in much smaller amount with asparagine as in TABLE 2. The fact that marked increase in the asparagine level of the medium did not abolish the requirements for the other amino acids but increased the growth in their presence is also in accord with the concept of asparagine serving as the principal nitrogen source.

Comparison reveals that all three strains were markedly deficient in the ability to synthesize phenylalanine with varying reductions in the capacity to form methionine and proline. The reduced ability to form tryptophane and tyrosine suggested by the preliminary experiments in the medium with low levels of glucose and asparagine was not significant when the higher levels were employed. Considerable differences in synthetic capacity have arisen between strains PS1 and PS2 which were derived originally from the single isolate employed previously (**6**). Inasmuch as the requirements of strain PS2 resemble those of the more recently isolated PS5, it is tempting to assume that it has retained the characteristics of the original isolate and that PS1 has undergone some change. It seems reasonable to suppose that the growth-promoting activity of yeast and compost extracts (**6**) can be attributed to the presence in them of thiamine, phenylalanine, methionine and proline. Further investigation would be necessary to establish this unequivocally, of course.

Requirements for specific amino acids have not commonly been found existing naturally in the Basidiomycetes (**9**) or lower fungi (**11**). However, Fries (**7**) reported that the growth of *Lentinus omphalodes* and several other Basidiomycetes was specifically promoted by tryptophane. Several aquatic Phycomycetes require methionine (**1**, **12**). Mutants specifically requiring phenylalanine, methionine, proline, or other amino acids have been artificially produced in *Neurospora* and several other fungi (**5**). Presumably, similar requirements may also originate in fungi in nature by gene mutation.

The ability of strains PS5 and PS2 to produce considerable growth upon addition of either methionine or phenylalanine is not easily interpreted. There may be production of some metabolic inhibitor whose action is reversed by either of these amino acids. Barrat and Ogata (**3**) and Barrat *et al.* (**2**) suggested this as the explanation for the requirement of a *Neurospora crassa* mutant for leucine or aromatic amino acids. Volcani and Sarid (**17**) found that the inhibition of the growth of *Escherichia coli* by dl-ethionine, dl-methionine sulfone and dl-methionine sulfoximine was reversed by dl-methionine, dl-phenylalanine or l-tryptophane. This may also indicate that methionine participates in the synthesis of phenylalanine. Addition of either of these amino acids might then be expected to compensate for a reduced ability to synthesize methionine and hence phenylalanine.

In addition to the inability of Treschow's (**16**) basal medium to supply these amino acid and vitamin requirements, it is apparent that the amounts of glucose and asparagine were not adequate to allow the maximum growth response to be observed in the presence of the former substances. A number of factors were no doubt responsible for this—e.g., strain differences, the smaller amounts of growth occurring in Treschow's (**16**) experiments and the culture of the organism at a constant temperature of 25° C compared to Treschow's (**16**) use of a variable and probably somewhat lower room temperature. Although a much more detailed analysis along the lines followed by Treschow (**16**) would be desirable to establish the optimal composition of the medium for growth in the presence of the amino-acid-vitamin mixture, the media with higher glucose and asparagine levels here employed probably approach this optimal composition.

SUMMARY

The amino acids phenylalanine, methionine and proline have been found to promote the growth of several strains of the common cultivated

mushroom, *Agaricus bisporus*, to varying degrees. Phenylalanine and methionine were the most active for two strains while phenylalanine and proline were the most active for the other strain tested. The presence of thiamine was necessary for an appreciable growth response to these amino acids. Approximately optimal levels for the glucose, asparagine and inorganic salts present in the medium have been determined in relation to the growth response to the amino-acid-vitamin mixture.

ACKNOWLEDGMENT

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AN ENTEROBRYUS FROM THE MILLIPED BORARIA CAROLINA (CHAMBERLIN)[1]

ROBERT W. LICHTWARDT

(WITH 20 FIGURES)

A previously undescribed species of *Enterobryus* has been found living within the hindgut of the milliped *Boraria carolina*.[2] This fungus, belonging to the order Eccrinales, lives attached to the chitinous lining of the milliped gut and to the cuticula of nematodes inhabiting the gut. It produces some spores of particular interest which have not been described heretofore, and has some features that further substantiate the belief that a commensal relationship exists between the Eccrinales and their hosts. In spite of the apparent non-parasitic nature of the fungus, attempts to culture this species outside of the milliped host were not successful.

MATERIALS AND METHODS

A colony of the handsome black and yellow milliped *Boraria carolina* was discovered living under the soil on a path of the Collins Creek Trail in the Smoky National Forest in North Carolina, and over one hundred individuals in various stages of maturity were collected. This find was supplemented by a few individuals from the Highlands Plateau. Preliminary studies were carried out at the Highlands Biological Station, but the majority of the millipeds were transported to the University of Illinois and kept alive in special containers in the mycology laboratory where they were dissected and studied during subsequent months.

The techniques employed in observing living and fixed material were substantially those described in a previous paper (Lichtwardt, **1954**).

[1] This paper is based upon a portion of a Ph.D. thesis submitted to the University of Illinois in 1954. I am greatly indebted to Dr. Leland Shanor who supervised this research, and also thank Prof. Thelma Howell, Executive Director, and the Highlands Biological Station for a grant from the Col. and Mrs. John S. Sewell Research Fund during the summer of 1952.

[2] I am grateful to Richard F. Hoffman for identifying specimens of this milliped.

OBSERVATIONS

Enterobryus borariae sp. nov.

Hyphis sterilibus plerumque rectis, basi vel apicibus aliquando curvis; saepius minus quam 5 mm longis, 3.5–50 μ diam. Cytoplasmate hyalino. Retinaculo plerumque cum orbi basali, usque ad 100 μ long., 6–12 μ diam. et quam hyphae angustiore. Sporangiosporis (typo A) circa 45–170 × 8–32 μ, ex foramine laterali ad terminum sporangii proximum emergentibus. Sporis multinucleatis (typo E) raris, circa 50 × 10 μ. Sporis uninucleatis (typo F) 9–16 μ long. et 9–18 μ lat. Sporangiosporis filiformibus (typo G) 90–150 μ long. et circa 3.5 μ lat. Sporis pyriformibus (typo H) sub velamine intestini sitis, 35–83 × 20–48 μ. Sporis ellipsoideis (typo I) circa 24 × 7 μ.

Vegetative hyphae predominantly straight, or curved at the proximal or distal end; usually less than 5 mm in length, diameter typically 8–18 μ with extremes ranging from 3.5–50 μ. Cytoplasm hyaline. Holdfast usually with a basal disk; holdfast stalk up to 100 μ in length, with long stalks showing prominent longitudinal striations; stalk width usually 6–12 μ, and narrower than attached hyphae. Sporangiospores (type A) abundantly produced, about 45–170 × 8–32 μ, emerging from a lateral pore at the proximal end of the sporangium. Multinucleate spores (type E) rare, about 50 × 10 μ. Uninucleate sporangiospores (type F) abundantly produced, 9–16 μ long by 9–18 μ wide. Filiform sporangiospores (type G) 90–150 μ long by approximately 3.5 μ wide, with tapered ends. Pyriform spores (type H) located beneath lining of hindgut, 35–83 × 20–48 μ, average size 50 × 35 μ. Ellipsoid spores (type I) about 24 × 7 μ.

Attached to the lining of hindgut of the milliped *Boraria carolina* (Chamberlin). Hyphae also attached to the cuticula of nematodes inhabiting hindgut of host. *R. W. Lichtwardt H-17-12.* TYPE.

The hyphae of *Enterobryus borariae*, like hyphae of many other species of Eccrinales, varied considerably in their dimensions. The length of the hyphae usually was not more than 5 mm, and the longer hyphae as a rule were the widest. The variability in width was especially noticeable in this species, ranging from 3.5 μ among some of the anterior hyphae, to as much as 50 μ farther back. The hyphae showed no definite spiraling, but the proximal or distal ends occasionally were gently arched.

Boraria carolina frequently is highly infected with nematodes. The number of nematodes may be as great as four or five dozen in one host, especially in the larger, female millipeds. The anterior portion of the milliped hindgut, the ileum, which is narrowed by a sphincter muscle, was found to be so packed with nematodes in some individuals that it appeared as though all solid material would be occluded. In fact, the

guts of several millipeds, when dissected, released living nematodes whose normally straight, rigid tails apparently were bent permanently in one or two places because of confining conditions. Perhaps this species of milliped contains more nematodes per individual, on the average, than any other which the writer has dissected, with the possible exception of *Spirobolus americanus*.

As in *S. americanus*, the nematodes living within the hindgut of *B. carolina* frequently may be found with hyphae attached to their cuticula (FIGS. 3, 4). It has been noted previously (Lichtwardt, **1954**) that in *E. elegans* from *Spirobolus* there is no visible penetration of the nematodes by the hyphae attached to them, either by the holdfast itself or by rhizoids arising from the holdfast. The same observation holds

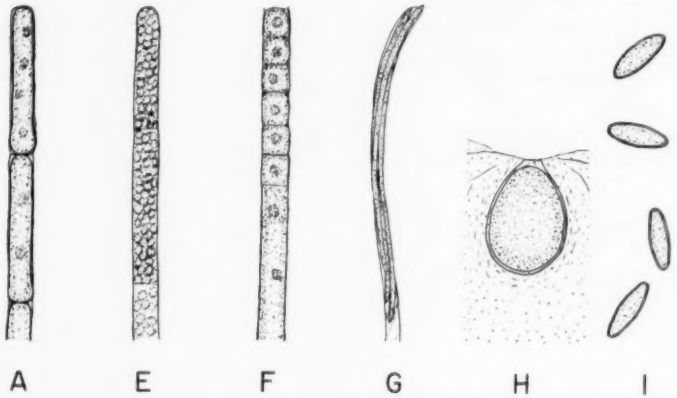
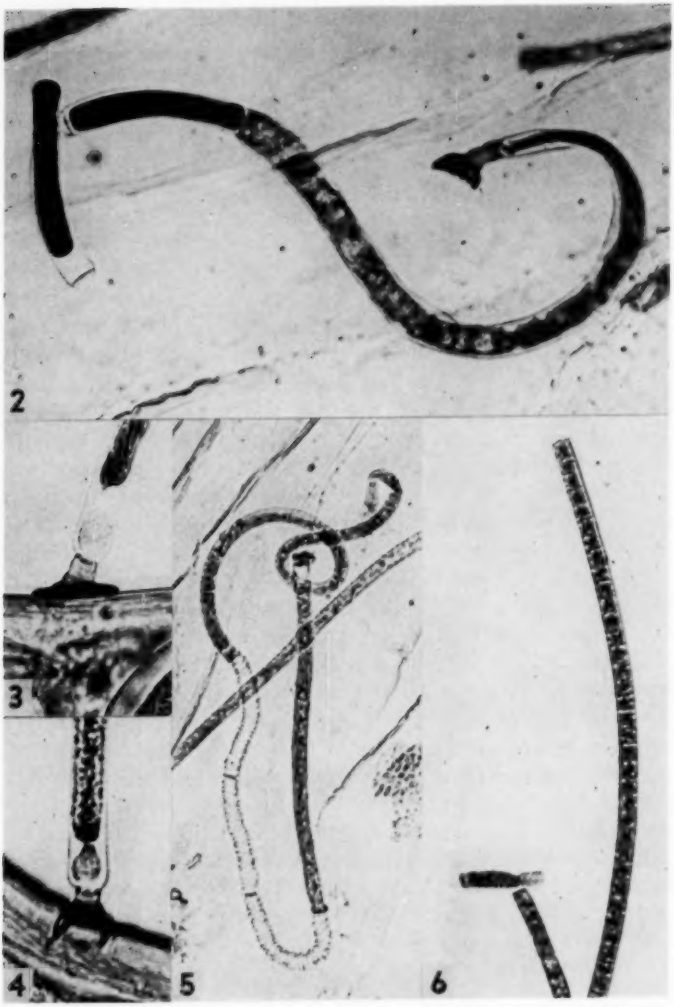


FIG. 1. Spore types of *Enterobryus borariae*, all × 400. A. 4-nucleate sporangiospore. E. Multinucleate spores. F. Uninucleate sporangiospores. G. Filiform sporangiospores. H. Pyriform spore. I. Ellipsoid spores.

true in hyphae of *E. borariae* that are attached to nematodes. The holdfasts that anchor the hyphae to the cuticula of the nematodes are similar in shape to some of the holdfasts attached to the gut lining, though the basal disks of the holdfasts on the nematodes sometimes are slightly larger in diameter, possibly to attach the thallus more firmly to the motile nematodes.

Two types of spores commonly are produced by hyphae of *E. borariae*. The first is a sporangiospore (FIG. 1) which has been referred to as type A (Lichtwardt, **1954**) and which, in this species, is usually 4-nucleate at maturity. This spore type has been observed in all species



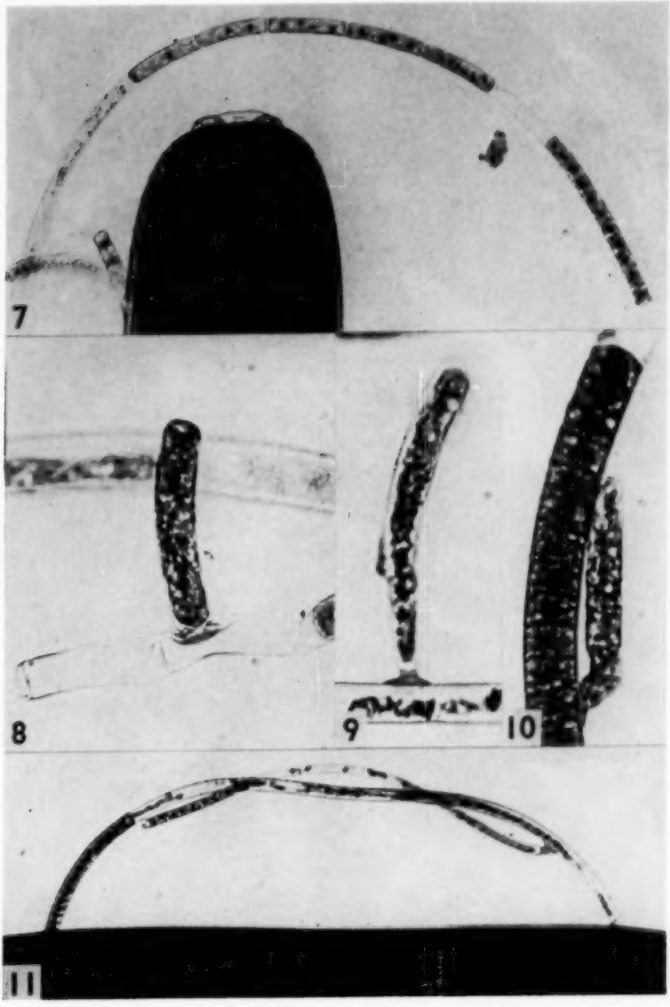
FIGS. 2–6. *Enterobryus borariae*. 2. Spore of type A emerging from the proximal, lateral end of its sporangium, × 550. 3. Disk-like holdfast attached to the cuticula of a nematode, × 600. 4. Holdfast appearing claw-like and giving the illusion of penetrating the cuticula, the result of bending of the nematode body, × 600. 5. Hypha with an elongate spore of type A and three empty, shrunken sporangia, × 250. 6. Hyphae bearing spores of type A (one emerging) and type F, × 250.

of *Enterobryus* that I have studied, and it serves as a means of reinfecting the same individual host. The spores escape from the sporangium through a lateral pore or tear at the proximal end of the sporangium (FIGS. 2, 6).

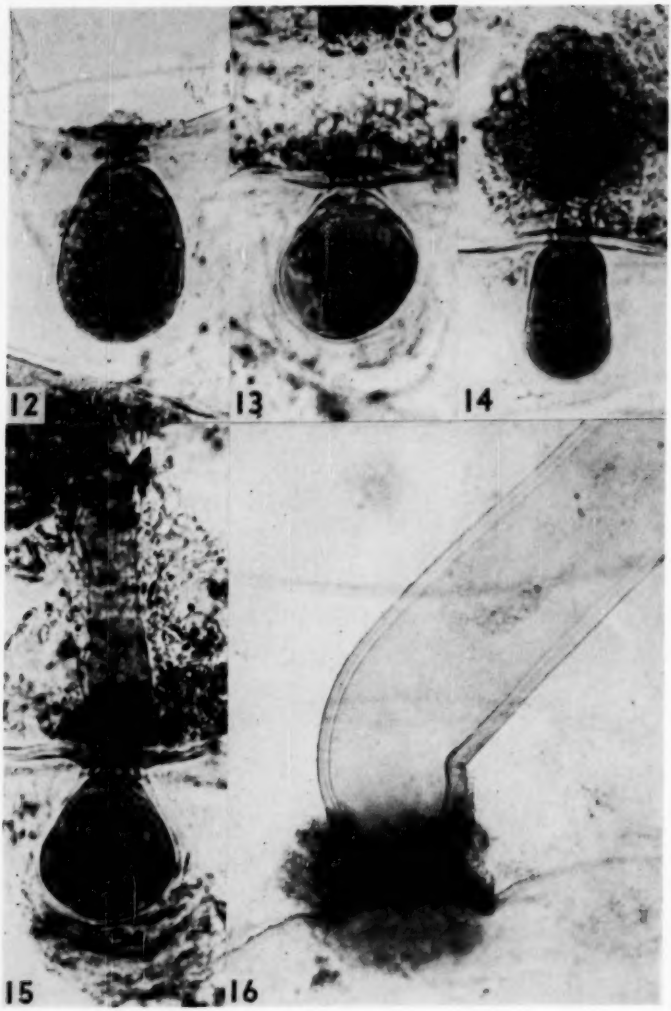
The second common spore produced by *E. borariae* is a uninucleate sporangiospore, referred to as type F (FIGS. 1, 6), also present in many other species of *Enterobryus*. The process of formation of this spore is similar to that of type A, but the sporangium is somewhat isodiametric and the single nucleus enclosed therein does not divide during formation of the spore. The uninucleate sporangiospore does not appear to be involved in reproduction of hyphae within the same host, and it has been suggested (Lichtwardt, **1957**) that this spore type may function in infecting other hosts. Spore type F thus seems to differ from spore type A both morphologically and functionally.

A thallus normally produces only one type of spore, but an interesting and unique condition was observed in a few hyphae of *E. borariae* in which spores of type A and type F were present in the same hypha. FIG. 7, for instance, shows a hypha which began producing type F spores (which are terminal), then switched to producing the elongate type A spores. The reverse situation also has been seen; that is, production of type A spores followed by the formation of type F spores. This is evidence that any hypha capable of producing one of these spore types is potentially capable of producing the other was well. It is conceivable that these abnormal hyphae with both spore types might have been subjected to certain environmental changes that affected spore production.

Another interesting situation of some rarity among the Eccrinales, which has been seen occasionally in *E. borariae*, concerns the fixation of spores to a particular substratum. It is stated frequently in the literature on the Eccrinales and the Trichomycetes that a hypha will grow only on a chitinous substratum, be it the lining of the gut, the cuticula of nematodes, or, in the case of certain ectophytes among these fungi, the exoskeleton of Arthropods. Yet, I have found spores and hyphae of *E. borariae* attached to other sporulating and non-sporulating hyphae of the same species, and even attached to empty sporangia (FIGS. 8–11). As has been observed on normal substrata, the holdfast of the attached thallus in these instances does not penetrate the fungal substratum. These observations indicate that spores are not totally discriminating in selecting the substratum upon which they will germinate, grow, and sporulate; and, more importantly, it supplements other evidence which points to a lack of true parasitic existence of the fungi within the gut.



FIGS. 7–11. *Enterobryus borariae*. 7. A single hypha bearing both spores of type F (terminal) and type A, separated by an empty sporangium of spore type A, × 250. 8. Spore attached to an empty sporangium, × 600. 9. Germinating spore attached to a vegetative hypha, × 600. 10. Germinating spore attached to sporangia, × 600. 11. A sporulating hypha attached to another sporulating hypha, × 150.



FIGS. 12–16. *Enterobryus hororiae*, all × 600. 12. Pyriform spore (type H), which was located between lining and epithelium of hindgut, attached to chitinous lining. 13. Germinating spore penetrating into gut cavity by means of a nipple-like structure. 14. Formation of hypha in gut cavity (covered by bacterial growth). 15. Further stage of formation and maturation of hypha. 16. Large, yellowed hypha that arose from spore of type H.

A most unusual reproductive body, designated as spore type H (FIGS. 1, 12–16), has been found in *Boraria carolina*, located *beneath* the chitinous lining of the middle and posterior part of the hindgut, but apparently not penetrating the tissues beneath the lining. The spores are generally pyriform, occasionally globose or elongate-pyriform, and are attached to the gut lining by means of a cap-like structure located near the narrower end of the spore and contiguous with the distinct wall which surrounds the spore. It has not been determined how these spores reach this position, nor has their origin been established, in spite of the fact that many millipeds have been found harboring them, and some individuals contained large numbers of these spores.

The pyriform spores have been found in various stages of germination. A spore penetrates the chitinous lining of the gut (FIG. 13) and produces a rounded nipple that looks superficially like the apophysis in certain eucarpic chytrids. From this structure arises the hypha (FIGS. 14, 15). Large amounts of debris and bacteria usually surround the hypha, and after it has elongated to a considerable extent, the pyriform body from which it arose shrinks and eventually disappears. Hyphae arising from these spores have been seen producing uninucleate sporangiospores of type F, similar to those produced by other hyphae in the same host.

The regions of the hindgut that produce spores of type H usually contain a number of thick-walled hyphae with short holdfasts which are believed to have arisen from spores of type H. FIG. 16 illustrates one of the largest of these. The bending near the base is more or less characteristic of the hyphae.

A few hyphae bearing multinucleate spores of type E (FIG. 1) have been found in *Boraria carolina*. These spores have been reported in *Enterobryus curyuri*, *E. apheloriae* (Lichtwardt, **1954**), and I have also seen them in a few other undescribed species of *Enterobryus*.

In a number of *Boraria carolina* individuals very narrow hyphae were found along the anterior region of the hindgut, attached to the gut lining by means of a slender holdfast, and producing slender, filiform spores with tapered ends. These spores are designated as spore type G (FIGS. 17–19). Careful examination indicates that these spores are formed singly in sporangia whose walls are so oblique that several of them may overlap in a hypha. The spores may be predominantly straight, though many of them are sinuous. The walls separating the sporangia are very thin and difficult to observe, a result in part of the slenderness of the hyphae that produce them. I would interpret these spores as being morphological modifications of spore

FIGS. 17–20. *Enterobryus bororiae*. 17. Narrow, anterior hypha producing spores of type G, × 250. 18, 19. Filiform spores of type G in sporangia, × 600. 20. Spore of type I germinating on lining of gut, × 600.

type A. They emerge from the end of the sporangium and become attached to the gut lining near where they were formed. I have found this spore type also in undescribed material collected in South America.

The sixth spore type, designated as spore type I (FIG. 1), was found in several hosts, but no hyphae have been discovered bearing this kind of spore. The spores are ellipsoid and germinate in the fashion of type A spores (FIG. 20). Spores of type I have been found in several other undescribed North American millipeds.

DISCUSSION

Variation within species of the Eccrinales is not unusual, though the degree of variation may be cause for concern among some biologists who are not familiar with the group. Yet, if one studies the variation in morphology within a species in terms of the habitat of these fungi and the biotic relationships which exist in this habitat, one notices that morphological variation in many species of Eccrinales is the rule rather than the exception. It is only necessary to define the limits of the variation, much as we define the morphological extremes in conidial shape and size in a fungus such as *Fusarium*, or the variation of spore types to be found within the life cycles of macrocyclic rusts.

There are two sets of major influences that seem to affect the commensal fungi of Arthropods. The first of these is the condition existing in the specific region of the hindgut which harbors the fungi. It can be demonstrated, for instance, that pH differences exist in different areas of the hindgut, and it is reasonable to expect that there will be differences in available foods, secreted products, and accumulated waste materials, from one region of the hindgut to another. Certain organisms such as protozoa, nematodes, and bacteria may be restricted to a greater or lesser extent in certain portions of Arthropod hindguts, and these regional concentrations of organisms indicate that there are physiological differences in habitat within the hindgut. These organisms, in turn, probably have an influence upon the growth of the commensal fungi, either adverse or beneficial. Some members of the Eccrinales are restricted to particular areas of the hindgut, while others may be found throughout the hindgut, though perhaps more concentrated in one area. It is my observation, after studying many species of *Enterobryus* in millipeds, that in general those fungi which show a greater distribution within the hindgut also show greater variability in morphological detail, and this variability may well be due in part to environmental differences along the length of the gut. *Enterobryus borariae* would seem to be

an excellent example of this situation, illustrated in part by its very slender hyphae in the anterior hindgut, ranging to the very wide, thick-walled hyphae in the middle and posterior portions of the hindgut.

The second influence upon the development of the Eccrinales is due to the cyclic or developmental changes which occur in the host, particularly those involving molting. For instance, many species of Eccrinales are reported by European workers to produce certain heavier-walled spores at the time the host molts, while between molts the developing hyphae produce spores of other types. This suggests that a given vegetative hypha, which already is growing within the gut, is capable of producing spores of either one type or another, the type it does produce perhaps depending upon the age of the hypha and the degree of influence of the immediate environment upon the hypha. The unusual instances of single hyphae producing two types of spores cited earlier in this paper, and illustrated in FIG. 7, are probably another manifestation of how the changes in the environment in the host gut may affect fungal development. Another example has been described in *E. elegans* from the milliped *Spirobolus americanus* (Lichtwardt, **1954**), in which the fungal hyphae attached to the gut lining produce a modified type of spore just prior to molting of the host, these spores anchoring themselves in great numbers to the nematodes in the gut cavity, the nematodes in turn serving as a temporary substratum to maintain thalli of the fungus species in the milliped while the gut lining is shed with the molt.

Frequently it is not possible to determine which of the above two environmental influences in the gut (i.e., locational or cyclic) seems to have the greater effect on the development and sporulation of the thalli. There is also a third influence on the morphology of species of Eccrinales, one which results in the formation of abnormalities such as grotesquely-shaped holdfasts and aborted or ill-formed spores, and it is necessary to distinguish forms with slight abnormalities from structures resulting from more normal variations. Such abnormalities probably arise from adverse environmental influences in the host and are comparable to what we observe among many laboratory cultures or in nature when conditions become unfavorable for normal development of the fungus. In millipeds, the fungal abnormalities seem to be most frequent in individuals that are most heavily infected, with consequent crowding of the thalli.

SUMMARY

Enterobryus borariae, a new species of Eccrinales from the hindgut of the milliped *Boraria carolina* (Chamberlin), is described and illus-

trated. Unusual instances are reported of spores and hyphae attached to other thalli of the same species, and of hyphae producing more than one type of spore. Among the various spore types produced by the species, in several millipeds an interesting spore-like body was found, located beneath the lining of the gut, that penetrated the lining and produced sporulating hyphae within the gut cavity. Variation within species of Eccrinales is discussed, and some of the factors responsible for this variation are suggested.

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SUSCEPTIBILITY OF CLARKIA TO SYNCHYTRIUM FULGENS[1]

GEORGE C. HARTMANN

(WITH 22 FIGURES)

Synchytrium fulgens is a long-cycled species which produces prosori, sori, sporangia, zoospores, and resting spores and parasitizes a large number of onagraceous species. In a study of the host range of this fungus, gall development, and host reaction to infection, Sinski (**1955** & **1956**) reported that some species of *Clarkia* are immune or resistant to infection and development of the parasite. His tests were limited, however, because he had only a few species of *Clarkia* available. I have been able to extend the tests begun by Sinski because of the generous gift of seeds of a large number of species and subspecies by Dr. Harlan Lewis of the University of California. Inasmuch as the chromosome numbers and karyotypes of these species are known, they provide the opportunity to determine whether or not the susceptibility of *Clarkia* is correlated with such factors. This paper, concerned with host susceptibility, size and shape of galls induced, and type of parasite development, embodies the results of my study.

The fungus used in this study was collected by B. T. Lingappa and maintained on *Oenothera biennis* in the greenhouse. This species has been described by Karling (**1954c**), Sinski (**1956**) and Lingappa (**1957**) as *S. brownii*, but extensive investigations by Karling (**1956**, **1958**) of *S. fulgens* indicate that these species are identical. Accordingly it is referred to as *S. fulgens*. Host seedlings were inoculated by the methods used by Karling (**1954b**, **1957**), Sinski (**1955** & **1956**) and Lingappa (**1957**) for *S. australe*, *S. decipiens*, and *S. fulgens*. Each host plant was inoculated at least twice at intervals of forty-eight hours and examined periodically under a stereo-microscope for evidence of infection. On susceptible plants, the sori and sporangia matured in ten to sixteen days, depending upon seasonal greenhouse temperature. Sporangia from each susceptible host were tested for the production of viable

[1] This study has been supported by a David E. Ross Fellowship from the Purdue Research Foundation. I am sincerely grateful to Dr. J. S. Karling for his guidance in this study and to Dr. Harlan Lewis for the seeds of *Clarkia*.

zoospores, and portions of infected leaves with mature sporangia were fixed, embedded, sectioned, and stained in order to examine gall structure and host reaction. All measurements given in the following tables were made from these stained sections. The size of the induced gall was determined by measuring the height of the gall and then the width on a line perpendicular to the height. The degree of gall protrusion on the host was determined by the difference between the width of a leaf through the gall and the width of an apparently normal portion of the leaf.

HOSTS

TABLE I lists the species tested for susceptibility. The + and − signs in column 2 indicate infection or lack of infection, respectively.

TABLE I

SUSCEPTIBILITY OF CLARKIA TO SYNCHYTRIUM FULGENS

Species	Susceptibility	Haploid chromosome number	No. of plants inoculated	No. of plants infected	No. of galls on each plant
C. amoena subsp. *huntiana*	+	7	6	4	20, 16, 6, 4
C. biloba subsp. *biloba*	+	8	4	2	19, 12
C. biloba subsp. *brandegeae*	+	8	5	3	26, 3, 6
C. bottae	+	9	4	3	63, 8, 8
C. cocinna	+	7	3	2	180, 28
C. cylindrica	+	9	9	3	32, 6, 1
C. dudleyana	+	9	3	2	27, 12
C. lassenensis	+	7	4	4	92, 2, 15, 4
C. mildrediae	+	7	1	1	3
C. modesta	+	8	1	1	29
C. purpurea subsp. *purpurea*	−	26	17	0	0
C. rubicunda subsp. *rubicunda*	+	7	9	3	27, 12, 1
C. unguiculata	+	9	2	2	5, 2
C. williamsoni	+	9	4	4	40, 19, 15, 1

As indicated in TABLE I, galls were produced on all species except *C. purpurea* subsp. *purpurea*. Seventeen plants of this species were inoculated (four of them seven times) but no infection occurred. Mature sori were not found on *C. unguiculata*, but further study of this problem was not feasible because of the failure of this species to grow in the greenhouse.

Viable zoospores were produced by the sporangia of *C. amoena* subsp. *huntiana*, *C. biloba* subsp. *biloba*. *C. biloba* subsp. *brandegeae*, *C. bottae*, *C. concinna*, *C. cylindrica*, *C. dudleyana*, *C. lassenensis*, *C. modesta*, *C. rubicunda* subsp. *rubicunda*, and *C. williamsoni*. *C. mildrediae* produced mature evanescent sori but viable zoospores from these sporangia were not observed in water mount.

Resting-spore galls were produced on *C. amoena* subsp. *huntiana*, *C. biloba* subsp. *biloba*, *C. biloba* subsp. *brandegeae*, *C. concinna*, *C. lassenensis*, *C. rubicunda* subsp. *rubicunda*, and *C. williamsoni*. I observed only one resting gall on the last species but I have included its measurements in TABLE III.

In addition to the development of normal galls, sori, and sporangia noted above, chronic abortive reactions of the type described by Karling (**1954b**) and Sinski (**1956**) occurred on *Clarkia amoena* subsp. *huntiana*, *C. biloba* subsp. *biloba*, *C. biloba* subsp. *brandegeae*, *C. bottae*, *C. cylindrica*, *C. concinna*, *C. dudleyana*, *C. lassenensis*, *C. modesta*, *C. rubicunda* subsp. *rubicunda*, *C. williamsoni*.

TABLE II

MEAN EVANESCENT-GALL SIZE AND DEGREE OF LEAF PROTRUSION

Species	Gall		Area	h-w index	Sorus		Area	h-w index	Protrusion
	ht.	wd.			ht.	wd.			
C. amoena subsp. *hunt.*	85.5μ	102.3μ	6750μ²	.84	67.6μ	75.8μ	4020μ²	.91	21.2μ
C. biloba subsp. *bil.*	90.2μ	105.2μ	7460μ²	.86	72.4μ	76.7μ	4420μ²	.93	39.8μ
C. biloba subsp. *brand.*	91.7μ	121.9μ	8760μ²	.75	65.3μ	77.1μ	3950μ²	.84	34.9μ
C. bottae	82.6μ	97.5μ	6300μ²	.85	64.7μ	72.6μ	3690μ²	.89	28.8μ
C. concinna	116.5μ	117.2μ	10,710μ²	.99	84.7μ	79.4μ	5270μ²	1.07	65.3μ
C. cylindrica	62.6μ	83.4μ	4100μ²	.75	50.5μ	66.0μ	2620μ²	.77	29.1μ
C. dudleyana	80.0μ	172.5μ	10,830μ²	.47	67.1μ	64.5μ	3405μ²	1.04	33.8μ
C. lassenensis	80.7μ	98.9μ	6260μ²	.81	65.6μ	77.1μ	3961μ²	.85	sunken below leaf surface
C. modesta	87.3μ	97.1μ	6660μ²	.90	66.5μ	67.3μ	3820μ²	.99	49.1μ
C. rubicunda subsp. *rubicunda*	107.6μ	117.5μ	9940μ²	.92	76.4μ	72.6μ	4360μ²	1.05	36.2μ
C. williamsoni	81.0μ	91.0μ	5790μ²	.89	69.1μ	69.2μ	3860μ²	1.00	18.6μ
C. mildrediae	35.0μ	105.0μ	2880μ²	.33	24.5μ	70.0μ	1379μ²	.35	17.5μ
C. unguiculata	150.0μ	189.2μ	22,280μ²	.79	99.7μ	122.5μ	9590μ²	.81	100.2μ
*O. biennis**	136.7μ	107.5μ	12,300μ²	1.23	87.7μ	75.2μ	5050μ²	1.2	

* Mean gall size of *S. fulgens* on *O. biennis* from Sinski (**1955**).

Acute abortive reactions as reported by Karling (**1954b**) for *S. australe* occurred on *C. biloba* subsp. *biloba*, *C. biloba* subsp. *brandegeae*, *C. rubicunda* subsp. *rubicunda*, and *C. williamsoni* (FIGS. 19, 20).

Lewis and Lewis have stated that the original basic chromosome number of *Clarkia* is 7. Genomes of 8, 9, and 12 are probably the result of the addition of supernumerary chromosomes, and genomes above 12—i.e., 14, 17, 18, and 26—are the results of polyploidy (Lewis–Lewis, **1955**). Of the species available to me, *C. purpurea* subsp. *purpurea* was the only one with a high chromosome number that would grow in our greenhouse. As TABLE I shows, those species with haploid chromosome numbers of 7, 8, and 9 were readily infected, whereas *C. purpurea* subsp. *purpurea* was immune.

DISCUSSION

The foregoing data indicate that the host range of *S. fulgens* on *Clarkia* is considerably wider than was previously known. On all species, the galls induced are predominantly composite, as described by Karling (**1954c**). The mean gall size of the evanescent sorus tends to vary somewhat from species to species. The species also differ in the extent to which the galls protrude on the leaf surface. Although the size of the resting gall varies, the shape is spherical or subspherical in all species observed. In general, resting-spore galls do not protrude but are level with the leaf surface. Although abortive growth was found in almost all species, *S. fulgens* may also produce mature sporangia on the same species. Some indication that plants with a haploid chromosome number of 7, 8, or 9 may be more susceptible than polyploid species was observed.

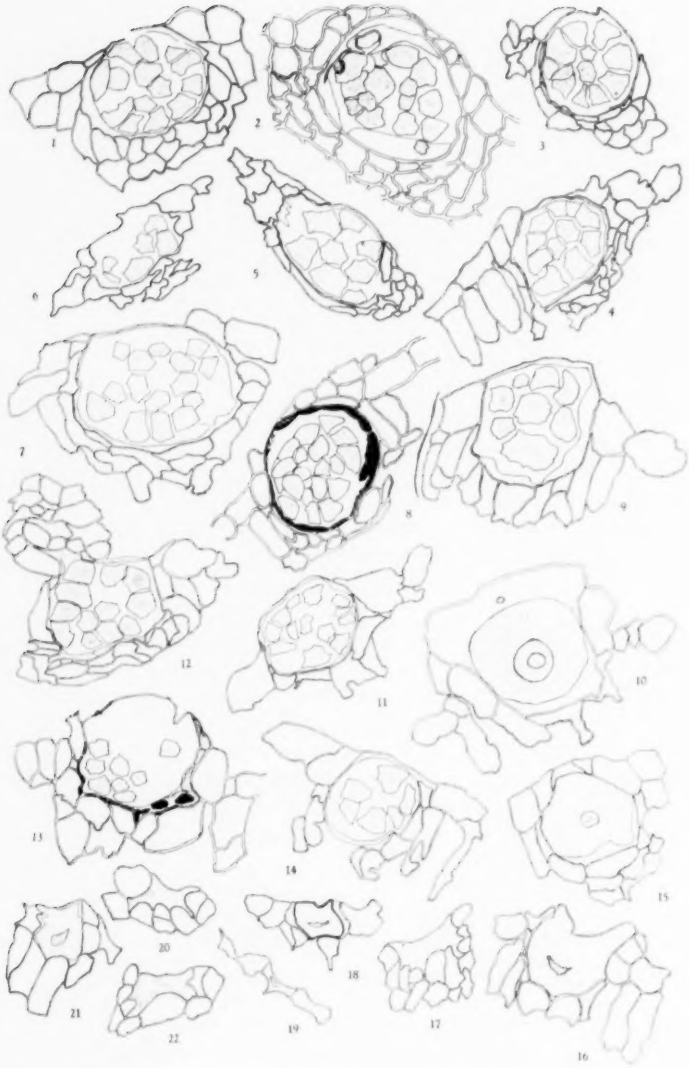
Relative susceptibility of *Clarkia* was investigated by counting the number of galls produced per plant (TABLE I). A number of factors, such as amount of inoculum, amount of moisture present, and length of time inoculum is applied to the host, affects the number of galls produced. However, by attempting to standardize these factors, I hoped some gross indications of susceptibility could be discerned. The number of plants I was able to grow was not large enough to make the results statistically valid, hence I have not shown a standard deviation for the mean number of infections per plant. The data do seem to indicate that certain species, such as *C. lassenensis*, *C. bottae*, and *C. concinna*, are more susceptible than such species as *C. unguiculata* and *C. mildrediae*.

As shown in TABLE II the gall and sorus size varies from species to species. In order to compare the size of the galls and sori produced on each species, the height and width were assumed to be the minor and major axes of an ellipse and the area of the ellipse was calculated. A height-width index was calculated for each species in order to compare the shapes of the galls and sori.

$$\frac{\text{height of gall}}{\text{width of gall}} = \text{G (height-width ratio of gall)}$$

$$\frac{\text{height of sorus}}{\text{width of sorus}} = \text{S (height-width ratio of sorus or resting spore)}$$

The largest galls were produced on *C. unguiculata*, *C. dudleyana*, *C. concinna*, and *C. rubicunda* subsp. *rubicunda*; the smallest were produced on *C. cylindrica*, *C. williamsoni*, and *C. mildrediae*. The galls on the remaining species fell within an intermediate range of 6200–8700 μ^2 (TABLE II). The galls on *O. biennis* were larger than the galls pro-



FIGS. 1–22. Galls of *Synchytrium fulgens*. FIGS. 1–14 represent mature sori on various hosts. FIG. 1. on *O. biennis*. FIG. 2. on *C. biloba* subsp. *brandegeae*. FIG. 3. on *C. modesta*. FIG. 4 on *C. dudleyana*. FIG. 5. on *C. cylindrica*. FIG. 6. on *C. mildrediae*. FIG. 7. on *C. rubicunda* subsp. *rubicunda*. FIG. 8. on *C. amoena*

duced on all species of *Clarkia* except *C. unguiculata*. The sori produced within the galls showed less of a range. The largest sori were produced on *C. unguiculata;* sori on the remaining species varied from 1300 μ^2–5200 μ^2. Nine of these species were in the range 3000 μ^2–4500 μ^2. The sori on *O. biennis* were larger than all of those on all *Clarkia* species except *C. unguiculata* and *C. concinna*.

A height-width ratio of one indicates a circular gall or sorus. A ratio larger than one indicates an upright ellipse [0], and a ratio less than one a flattened ellipse [⊂]. The evanescent galls vary from elliptical as in *C. mildrediae*, to almost circular as in *C. concinna*, *C. modesta*, and *C. rubicunda* subsp. *rubicunda*. In *C. mildrediae* the sori tend to be a flattened ellipse but in the remaining species are upright or circular. Both the galls and sori on *O. biennis* tend to be circular or slightly upright.

C. rubicunda subsp. *rubicunda* and *C. concinna* produce the largest resting galls. In *C. amoena* subsp. *huntiana* the gall is intermediate but the resting spore is quite large (2620 μ^2). The largest resting spores of all species are produced on *C. concinna*. Both the resting galls and sori of *O. biennis* are quite small. The galls on *O. biennis* are approximately the same size as the smallest *Clarkia* galls—those on *C. lassenensis* and *C. biloba* subsp. *brandegeae*. Except for the resting galls on *C. amoena* subsp. *huntiana*, the resting galls and spores on all species of *Clarkia* and *O. biennis* tend to be circular (TABLE III).

The degree of gall protrusion on some species was quite different from what had been previously observed on *Oenothera*, where the galls protrude only slightly (FIG. 1). On *C. lassenensis* (FIG. 12) either all the galls are sunken below the leaf surface or the top of the gall is level with the leaf epidermis. On *C. unguiculata*, *C. modesta*, and *C. concinna* a large part of the gall was above the surface of the leaf epidermis (FIGS. 3, 10, 13). In some cases almost the entire gall protruded from the leaf surface. Other species show a gall protrusion intermediate between these two extremes (FIGS. 2, 4–9, 11, 14). In other words, the

subsp. *huntiana*. FIG. 9. on *C. biloba* subsp. *biloba*. FIG. 10. on *C. unguiculata*. FIG. 11. on *C. bottae*. FIG. 12. on *C. lassenensis*. FIG. 13. on *C. concinna*. FIG. 14 *C. williamsoni*. Magnification of FIGS. 1-12, 14, × 700; 13, × 600. FIG. 15. Early chronic abortive thallus on *C. biloba* subsp. *biloba*. FIG. 16. Later stage of chronic abortive thallus on *C. amoena* subsp. *huntiana*, showing collapsed nucleus. FIG. 17. Chronic abortion on *C. cylindrica*, showing inward growth of cells surrounding the parasite. FIG. 18. Aborting resting gall on *C. biloba* subsp. *biloba*. FIG. 19. Acute abortion of thallus on *C. bottae*. FIG. 20. Acute abortion of thallus on stem of *C. cylindrica*. FIG. 21. Early acute abortion of thallus on *C. biloba* subsp. *biloba*. FIG. 22. Late chronic abortive state on *C. cylindrica*. Magnification of FIGS. 15–22, × 700.

galls produced on various hosts by inocula of *S. fulgens* from *O. biennis* varied in size, shape, and protrusion. This fact indicates, as Karling maintains (**5**), that gall formation is the result of the host-parasite relationship, and species distinction in *Synchytrium* should not be made primarily on the basis of gall formation.

My data yield no definite conclusions on the correlation between chromosome number and susceptibility to the parasite. But a trend toward some connection between chromosome number of 7, 8, or 9 and susceptibility is indicated. The only polyploid species I succeeded in growing, *C. purpurea* subsp. *purpurea* proved immune to the parasite.

As stated above, chronic abortive growth of the parasite was observed on most species. Reactions described by Karling (**1954b**) and characteristic of abortive growth—collapsed nuclei, degenerating cytoplasm, and inward growth of surrounding healthy cells—were all clearly

TABLE III

MEAN HEIGHT AND WIDTH OF RESTING GALLS AND SORI

Species	Gall		Area	h-w index	Resting spore		Area	h-w index
	ht.	wd.			ht.	wd.		
C. amoena subsp. *hunt.*	52.5μ	89.3μ	3870μ²	.58	59.5μ	56.0μ	2620μ²	1.1
C. biloba subsp. *bil.*	59.5μ	68.2μ	3200μ²	.87	64.7μ	63.0μ	3710μ²	.89
C. biloba subsp. *brand.*	53.5μ	54.5μ	2250μ²	.96	41.5μ	39.8μ	1280μ²	1.0
C. concinna	75.8μ	102.0μ	6090μ²	.75	56.1μ	67.9μ	2905μ²	.86
C. lassenensis	53.8μ	66.5μ	2800μ²	.81	40.2μ	48.1μ	1570μ²	.84
C. rubicunda subsp. *rubicunda*	113.1μ	74.7μ	6630μ²	1.5	45.8μ	47.0μ	1675μ²	.98
C. williamsoni	38.5μ	52.5μ	1580μ²	.73	38.5μ	31.5μ	950μ²	1.2
*O. biennis**	57.37μ	59.49μ	2680μ²	.97	45.32μ	38.5μ	1371μ²	1.2

* Mean gall size on *O. biennis* from Sinski (**1955**).

seen (FIGS. 15, 16, 17). Extrusion of the parasite as described on *Oenothera* by Sinski (**1956**) was not observed but the parasite seems to have simply degenerated (FIGS. 17, 22). Acute abortion reactions—i.e., where abortion occurs before the formation of a gall around the parasite by the host cell—were also observed as previously noted (FIGS. 19, 20, 21).

Gäumann (**1950**) has suggested that abortion is the result of a competition for an available amount of nutrient by the thalli of the fungus. Many of the abortive thalli were observed in confluent galls side by side with healthy mature sori. However, isolated abortive galls were commonly observed and in one plant of *C. cylindrica* where only one gall was produced the gall aborted after ten days' growth. Other factors beside parasite competition are probably important here. The careful exclusion of all insects from inoculated plants (a factor not possible in this study) is essential to the further investigation of this problem.

SUMMARY

1. Fourteen species and subspecies of *Clarkia* were inoculated with *S. fulgens*. Thirteen were found to be susceptible to the parasite and in eleven of these viable zoospores were produced.

2. The evanescent galls and sori on most species of *Clarkia* are somewhat smaller than the evanescent galls and sori on *Oenothera biennis*. In almost all species of *Clarkia* and in *O. biennis*, the galls and sori tend to be circular, slightly flattened, or upright. On only two species of *Clarkia* are the galls distinctly flattened. The resting galls and sori on *O. biennis* compare with the smallest of the resting galls, and sori produced on *Clarkia*. Except for one species (where the galls are flattened) all resting galls and sori produced on *Clarkia* and *Oenothera biennis* tend to be circular, and slightly flattened, or upright.

3. Slight evidence of a relationship between host haploid chromosome number and susceptibility to the parasite was observed.

4. A range of gall protrusion from a sunken condition to a highly protruding one was observed.

5. Abortion of the parasite was found in ten species but mature sporangia were also produced in all of these.

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NOTES ON FUNGI FROM NORTH-EAST INDIA. I. A NEW GENUS OF TUBERCULARIACEAE

V. Agnihothrudu [1]

(with 12 figures)

Comprehensive accounts of the principal diseases of tea in northeast India have been published in the past by Tunstall (**1940**) and Tunstall and Sarmah (**1948**). During the routine examination of diseased specimens of tea and its ancillary crops, the writer had occasion to isolate many interesting fungi and a few of them have already been reported elsewhere (Agnihothrudu and Barua, **1957a**, **b** and Agnihothrudu, **1958**). In view of the rich fungal flora, parasitic or saprophytic, associated with the tea gardens in northeast India, it is now proposed to record, describe or discuss fungi occurring in this area in a series of papers, of which the present is the first.

In the course of investigations on the fungi associated with withering floral parts of tea *Camellia sinensis* (L.) O. Kuntze, on three occasions an interesting tuberculariaceous fungus was isolated which was found to be hitherto undescribed. The fungus was first observed on dead calyces of tea flowers collected from the Tocklai Experimental Station campus, the flowers having been incubated on sterilized soil in glass trays under bell-jars. On two other occasions it was found to grow from petals plated on soil-extract agar. The fungus was seen in association with a variety of other fungi—*Pestalozzia* sp., *Fusarium semitectum* Berk. & Rav., *Pullularia pullulans* (de Bary) Berk., *Botrytis* sp., *Hormodendrum resinae* Lindau, *Cladosporium herbarum* Link ex Fr. and *Helicomyces* sp. Its ability to infect tea flowers has not been investigated.

The Fungus in Nature

The sporodochia are at first small, of the size of a pin-point, and are entirely superficial. They are white to begin with, turning pale dirty white on drying, typically sessile, measuring up to 1 mm in diameter and 300 μ in height when fully mature, and glabrous; the spore mass

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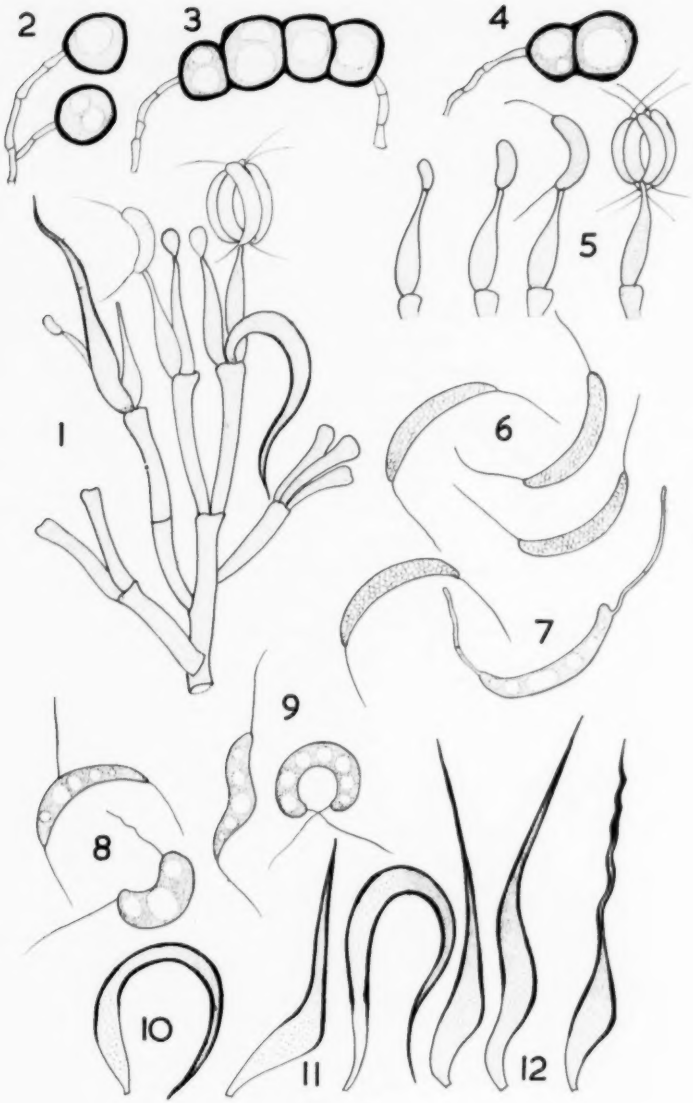
accumulates in long columns. The mycelium of the fungus is inter- and intracellular in the tissues of the flower and profusely branched, hyaline, septate, measuring up to 2.5 μ in diam., loosely interwoven to form a lax byssoid stroma at the base of the sporodochium.

The sporodochium consists of a dense aggregation of conidiophores which are repeatedly branched in an irregularly racemose fashion. The ultimate laterals are closely aggregated to form the fertile upper layer of the sporodochium. They are long, hyaline, slightly wider about the middle, narrower at the base and tapering to an almost pointed apex, measuring 9–15(–19) × 1.5–3.5 μ. Intermingled with the conidiophores are seen certain peculiar sterile setae that are hyaline, continuous, with a slightly bulbous base which is thin-walled, and prolonged above into a long pointed spine-like process which may be straight, undulate, uncinate or otherwise bent. The pointed part has a slightly thicker wall, appears refractive and bears minute verrucosities which impart a rough appearance to it. The setae separate easily in water mounts and measure 18–38(–48) × 1.5–4 μ. The conidia are borne singly at the apex of the conidiophores and are produced successively. They aggregate in a *Cephalosporium*-like head and are seen forming moist balls at the ends of the conidiophores as in the species of *Fusarium* that produce spores in pionnotes. The spores are fusoid-curved to falcate with granular contents when young, the apices subacute to obtuse, measuring 9–13(–18) × 1.5–3 μ, and bear at either end one long flexible seta measuring 3–8(–13) μ. The conidia are produced abundantly in nature.

The fungus was isolated from the floral parts by the dilution plate method, using acidified soil-extract agar.[2] The isolate was maintained with occasional transfers on soil-extract agar, potato-dextrose agar or sterilized bits of tea-stem until the present study was initiated a year ago. At that time monosporous cultures of the fungus were prepared and grown on potato-dextrose agar, soil-extract agar, Czapek-Dox agar, oat agar and sterilized tea-stem bits. Measurements, drawings of the conidia and other details were greatly facilitated by growing the fungus on a thin layer of nutrient agar in tubes. The setae were not very clear in water mounts but were distinctly seen when stained with Dorner's aqueous nigrosin in the same way as for bacterial negative mounts, a method used by the author for staining the appendages of *Starkeyomyces* (Agnihothrudu, **1956**).

[2] 1000 gm dry soil and 1 liter of water autoclaved for 30 minutes at 15 lb pressure, filtered through No. 1 Whatman filter paper; 15 gm agar added to the filtrate and made up to 1 liter, 0.2 gm $K_2H\ PO_4$ added as a buffer, sterilized at 15 lb pressure for 20 minutes, acidified with 25% lactic acid just before using.



FIGS. 1–12.

Thozetellopsis tocklaiensis. FIGS. 1–5 × 500; FIGS. 6–12 × 650.

CULTURAL CHARACTERISTICS OF THE FUNGUS

Potato-dextrose agar: Radial growth was slow; the diameter of the colonies grown from mass inoculum at 26–30° C reached 5 mm after 6 days and 15 mm after two weeks. The colonies after a week were pale with scanty aerial mycelium, white and cottony, collapsing with the aging of the culture. Sporodochia few and scattered. Color on the reverse Piping Rock (13 A 2).[3]

Tea-extract agar: Growth slow, 12 mm after two weeks. Aerial mycelium almost absent, sporodochia few and scattered, diffusing Shade of Tea (13 B 4) pigment.

Oat agar: Growth slow, aerial mycelium abundant, colony attaining 25 mm at the end of two weeks, white at first turning to Sand (13 B 2) or Nutria (15 A 3) with the aging of the culture. Reverse in shades of Smoky Brown (16 A 2) or Castor (16 A 8) and finally Biskra (16 A 12). Sporodochia almost absent.

Czapek-Dox agar: Growth 18 mm in two weeks, aerial mycelium sparse, white, collapsing, turning Deer plus (14 B 8) to Blondine (14 B 7). Reverse in shades of Fallow (12 B 5), Almond (13 B 6) and finally to Burnt Umber (15 A 12).

Soil-extract agar: Growth extremely slow, reaching 8 mm in two weeks, aerial mycelium almost absent, submerged mycelium Sandust (3 A 9) to Muscade plus (4 A 10). Reverse in shades of Flesh Blond (4 B 9) to Army Brown (6 B 10).

In all the nutrient media the sporodochia were very sparse and discrete. By repeated transfers the fungus failed to produce conidia on potato-dextrose, Czapek-Dox and oat agars. Chlamydospores were produced in abundance on all the media in the substrate mycelium. These are thick-walled, spherical, oval, intercalary or terminal and either single or in pairs or in chains measuring 13–16(–20) × 6–16 μ. The sterile setae present in the conidiophores were not produced in cultures which were less than a week old. On Czapek-Dox and oat certain abnormal spore forms were observed (FIGS. 8 and 9). Varia-

[3] The numerical designations corresponding to different colors in Maerz and Paul's *A Dictionary of Color*, McGraw-Hill, New York (1930) are included in parenthesis.

FIG. 1. Branched conidiophore with the ultimate laterals bearing conidia. Intermixed with the conidiophores are awn-shaped sterile setae. FIGS. 2–4. Chlamydospores from the substrate mycelium. FIG. 5. Stages in the conidium production. Conidia aggregated in *Cephalosporium*-like head. FIG. 6. Mature conidia. FIG. 7. Germinating conidium. FIGS. 8–9. Abnormal conidia from a culture on Czapek-Dox agar. FIGS. 10–12. Awn-shaped sterile setae.

tion in the dimensions of spores, setae, ultimate laterals of the conidiophores and the sterile setae of the fungus on different media is recorded in TABLE I.

Abundant sporulation was obtained on sterilized tea-stem bits. The fungus makes very fast growth; the aerial mycelium is scanty, white and on the second or third day of the culture it presents funiculose growth. The conidia also make their appearance now and are seen borne at the tips of the mycelial filaments that compose the funiculose growth. The ramose conidiophores appear after the fifth day when conidia are produced in greater abundance and they remain aggregated in slimy balls. It is observed that the conidia are free from any mucilaginous covering. The discrete sporodochia appear after the fifth day as minute specks and increase in size as well as number after the seventh or the eighth day. With the advance in age of the culture the stem bits are com-

TABLE I

DIMENSIONS OF CONIDIA, SPORE SETAE, ULTIMATE LATERALS OF THE CONIDIOPHORES AND STERILE SETAE OF THOZETELLOPSIS TOCKLAIENSIS ON DIFFERENT MEDIA

Measurements based on 100 readings

Medium	Conidia (μ)	Spore setae (μ)	Ultimate laterals of conidiophores (μ)	Sterile setae (μ)
Tea-stem bits	9.5–13(−16)×1.5–2.5	4.5–8(−13)	12–14.5(−18)×1.5–2.5	18–36(−48)×2.5–4
Tea-extract agar	10–13(−18)×1.5–2.5	3–8(−13)	10–13(−16)×1.5–2.5	18–32(−48)×1.5–4
Potato-dextrose agar	11–14(−16)×1.5–2.5	5–8(−13)	9–13(−16)×1.5–2.5	22–32(−45)×1.5–3.5
Oat agar	9–13(−16)×1.5–2.5	4–7(−13)	13–15(−19)×1.5–3	24–36(−48)×1.5–3
Czapek-Dox agar	11–14(−18)×1.5–3	5–8(−13)	13–16(−19)×1.5–3.5	26–38(−48)×2.5–4
Soil-extract agar	10–13(−18)×1.5–2.5	3–8(−13)	9–14(−19)×1.5–3	25–38(−48)×1.5–4

pletely covered with a uniform mat of sporodochia. The conidia are borne in pionnotes also. They are produced in such a profusion that they form long columns on the sporodochia in old cultures.

The spore is formed at the tip of the conidiophore as a small knob which gradually assumes the lunate shape of the mature spore and the setae at either end are formed when the spore is still attached to the conidiophore. The spores become displaced by the successive growth of the conidia from the apex of the conidiophore and remain aggregated in a *Cephalosporium*-like head at the tip.

The germination of the spore takes place readily in distilled water, producing one or two germ tubes from either extremity within 8 to 10 hours after incubation at the laboratory temperature (26–30° C). The spores cast off their setae in the process of germination. In some rare instances the spores were observed to form an equatorial septum at the time of germination.

The typically branched conidiophores with a basal byssoid stroma constituting the sporodochium and the unicellular setulate conidia characteristic of the fungus suggest that its position is undoubtedly in the amerosporous Tuberculariaceae of the Fungi Imperfecti.

In attempting to establish the identity of this fungus, it was compared with the genera of Moniliales which produce amerosporous, hyaline, setulate conidia.

The genera *Ellisiella* Saccardo (**1886**, p. 315) emend. Batista (**1956**), *Ellisiellina* Da Camara (**1949**) and *Ellisiopsis* Batista (**1956**) all bear dark-colored sterile setae and are characterized by conidia that are setulate at one end with the exception of *Ellisiellina biciliata* Da Camara (**1949**). *Pestalloziella* Saccardo (**1884**, p. *737*) has conidia with a ramose awn at the apex, while *Eriosporella* Hoehnel (**1916**) possesses three divergent setae. Therefore, it is obvious that the fungus occurring at Tocklai is entirely different from all these fungi.

The only genus that comes very near the fungus in question is *Thozetella* O. Kuntze (syn. *Thozetia* Berk. & Muell. (Berkeley, **1881**), non *Thozetia* Mueller) (Ainsworth and Bisby, **1954**), a monotypic genus. The fungus was described by its author as follows, "Sporodochium minutum, globosum; sporae hyalinae oblongae utrinque seta unica terminatae," and the type species *Thozetia nivea* Berkeley (syn. *Thozetella nivea* (Berk.) O. Kuntze) by "Snow-white, hyaline spores oblong, pointed at either end, and terminated with a single long bristle."

Unfortunately not much is known with any certainty about the genus. Cooke (**1892**) has mentioned this fungus in his *Handbook of Australian Fungi* and has given all the information contained in Berkeley's original diagnosis.

The only described species of the genus, *T. nivea*, was collected on putrefying wood from Queensland, Australia and has never been collected since then. Mr. E. W. Mason (*in litt.*) informs me that no type specimen of the fungus exists in the Commonwealth Mycological Institute or in the British Museum, nor is it in the Herbarium of the Royal Botanic Gardens, Kew. The description of the fungus in Saccardo (**1886**, p. 679) gives all the information contained in the generic and specific diagnoses. It is quite evident from the description of Berkeley that his fungus had conidia that were oblong. Nevertheless, von Hoehnel (**1909**) claimed that he had found the species *T. nivea* on bark in the Botanic Gardens at Buitenzorg and published a redescription of it. However, there is no particular reason to believe that Hoehnel had found the same fungus, in view of the meager description of the genus and its type species. But the spore measurements given by Hoehnel,

20–28 × 4 μ, rule out its identity with the fungus collected and cultured in our laboratory. Besides, Dr. M. B. Ellis (*in litt.*) draws attention to the fact that Hoehnel described a brown palisade of erect hyphae below the sporogenous cells, a structure not present in the fungus occurring on tea flowers here. Our fungus thus differs from *Thozetella* and is characterized by possessing a well-developed sporodochium and producing the spores in the same way as a pionnotes-forming *Fusarium*, the spores being distinctly fusoid-curved with obtuse to subacute ends bearing terminal setate; besides, the conidia are produced singly, successively and acrogenously; the conidiophores are well developed, irregularly branched, ending in subulate laterals intermingled with peculiar sterile setae. Hence, a new genus is proposed to accommodate the fungus; the name *Thozetellopsis* is chosen in order to indicate the generic relation with *Thozetella*.

THOZETELLOPSIS gen. nov.

Pertinet ad Fungos Imperfectos, ad Moniliales, Tuberculariaceas, Hyalosporas; sporodochia pulvino similia, ab inito superficialia, typice sessilia, nitentia, madida, glabra; mycelium sterile hyalinum, efformans stroma byssoides ad basin sporodochii; conidiophori bene efformati, ramosi, septati, hyalini, arcte aggregati ad efformandum seriem superficialem fertilem; cum conidiophoris intermixae sunt setae steriles aliquantum aristatae; conidia producta singula, acrogena atque successive ad apices conidiophororum insidentia, fusoideo-curvata ad falcata, levibus parietibus praedita, hyalina, continua, semel setulata ad utrumque apicem.

Fungus Imperfectus, Monidiales, Tuberculariaceae, Hyalosporae; sporodochia cushion-shaped, superficial from the first, typically sessile, glistening, moist, glabrous; sterile mycelium hyaline, forming a byssoid stroma at the base of the sporodochium; conidiophores well-formed, ramose, septate, hyaline, closely packed to form a superficial fertile layer; intermingled with the conidiophores are somewhat awn-shaped sterile setae; conidia produced singly, acrogenously and successively at the tips of the conidiophores, fusoid-curved to falcate, smooth-walled, hyaline, continuous, with one terminal seta at either end.

The specific name proposed, *Thozetellopsis tocklaiensis*, is after the Tocklai Experimental Station (Scientific Department of the Indian Tea Association) where the fungus was first collected and investigated by the writer.

Thozetellopsis tocklaiensis sp. nov.

Sporodochia pulvino similia, primo discreta tum evadentia aggregata in seriem continuam, ab initio superficialia, typice sessilia, alba, nitentia, madida, sub desicca-

tione evadentia sordide alba, 1 mm diam. 300 μ alta; mycelium sterile glabrum, exiguum, usque ad 2.5 μ crassum, hyalinum, laxe intextum ad efformandam seriem byssoideam ad basin sporodochii; conidiophori bene evoluti, longi, tenuibus parietibus praediti, irregulariter ramosi, ultimis ramulis arcte adpositis ad efformandam seriem superiorem fertilem sporodochii, longis, simplicibus, hyalins, continuis, cylindricis vel subulatis, 9–15(–19) × 1.5–3.5 μ; cum conidiophoris intermixtae sunt setae steriles, hyalinae, basi lata et tenuibus parietibus praedita, atque longo apice aristato crassis parietibus praedito, refractivo, aspero ob verruculas minutas, recto, tenuiter curvato, fortiter uncinato, undulato vel aliter curvato, 18–38(–48) × 1.5–4 μ; conidia producta acrogena, singula et successive ad apices conidiophororum, fusoideo-curvata vel distincte falcata, levibus parietibus praedita, hyalina, continua, contentis granularibus, 9–13(–18) × 1.5–3 μ, apicibus subacutis vel obtusis, longo setulo 3–8(–13) μ longo flexili ad utrumque apicem ornata.

Sporodochia cushion-shaped, discrete at first, becoming merged to form a continuous layer, superficial from the first, typically sessile, white, glistening, moist, turning dirty white with drying, measuring up to 1 mm in diam. and 300 μ in height; glabrous, sterile mycelium up to 2.5 μ thick, hyaline, loosely interwoven to form a byssoid layer at the base of the sporodochium; conidiophores well-formed, long, thin-walled, branched in an irregularly racemose fashion, the ultimate branches closely apposed to form the fertile upper layer of the sporodochium, long, simple, hyaline, continuous, cylindrical to subulate, measuring 9–15(–19) × 1.5–3.5 μ; interspersed with the conidiophores are certain sterile setae that are hyaline with a broad thin-walled base and a long pointed awn-like thicked-walled apex, refractive, rough from minute verrucosities, straight or slightly bent, strongly uncinate, undulate or otherwise bent, 18–38(–48) × 1.5–4 μ; conidia produced successively, singly, acrogenously at the tips of the conidiophores, fusoid-curved to distinctly falcate, smooth-walled, hyaline, continuous, with gramular contents, 9–13(–18) × 1.5–3 μ, extremities subacute to obtuse bearing a long flexible seta at either end 3–8(–13) μ in length.

The TYPE is represented by a single-spore culture isolated from the decaying floral parts of tea (*Camellia sinensis*), collected from the tea-seed-garden within the campus of Tocklai Experimental Station. Material of the type has been deposited in the Mycological Herbarium, Tocklai Expl. Station; Centraalbureau voor Schimmelcultures, Baarn, Holland; Commonwealth Mycological Institute, Kew, Surrey, England and the Herbarium Cryptogamie Indiae Orientalis, New Delhi, India.

ACKNOWLEDGMENTS

My thanks are due to the Director, Tocklai Experimental Station for permitting me to publish this paper and to Mr. K. C. Sarmah,

Mycologist, Indian Tea Association, for his encouragement. I am deeply indebted to Mr. E. W. Mason and Dr. M. B. Ellis of the Commonwealth Mycological Institute for examining the fungus and offering valuable suggestions, to Professor G. W. Martin of the State University of Iowa and to Miss Edith K. Cash of the Plant Industry Station, United States Department of Agriculture, Beltsville, Maryland, U.S.A., for critically reading the manuscript, and to Rev. Fr. Dr. H. Santapau, of the St. Xavier's College, Bombay, for rendering the Latin diagnosis.

SUMMARY

A hitherto unreported fungus isolated from the decaying floral parts of *Camellia sinensis* (L.) O. Kuntze is described and assigned to a new genus *Thozetellopsis*. The genus is distinguished by the production of sporodochia, fusoid-curved unicellular conidia with a filamentous seta at either end and by the peculiar awn-shaped sterile setae found interspersed with the conidiophores. The species has been designated, *Thozetellopsis tocklaiensis*.

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NOTES AND BRIEF ARTICLES

Haplomycosis in Japan and Africa[1]

Haplomycosis, a pulmonary mycosis of wild rodents and other small mammals, is caused by fungi of the genus *Haplosporangium*. The etiologic agent, first found in rodents in Arizona, was described and named *Haplosporangium parvum* by Emmons and Ashburn (**1942**). The species name may not be appropriate for strains of fungi that have been found in other geographical areas, but other names have not been proposed. Haplomycosis has since been found in western Canada by Dowding (**1947**), in many parts of western United States by Jellison (**1950**), in Korea by Jellison (**1954**), in England by McDiarmid and Austwick (**1954**) and by Tevis (**1956**), and in Norway by Jellison (**1956**).

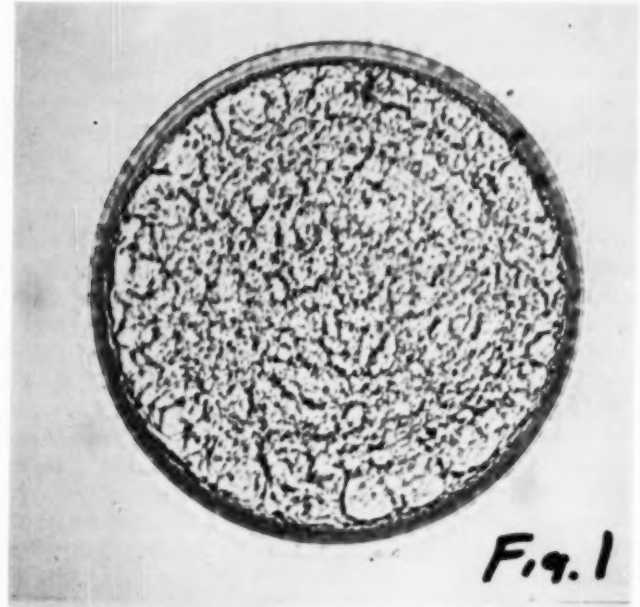
This mycosis can now be reported in two species of rodents from Japan and in a rodent and a carnivore from Africa after finding typical cysts in preserved specimens of lungs received for examination. This diagnosis is based on the microscopic appearance of tissue spherules in whole mounts and histologic sections. It is believed justified because of the considerable experience of the writer in the microscopic examination and culture of rodent lungs for *Haplosporangium*. Confirmation of these findings in each country by culture of the fungus would be very desirable but this must be done by someone with access to fresh specimens.

Through the courtesy of Dr. Masami Kitaoka, Department of Viral and Rickettsial Diseases, National Institute of Health, Tokyo, I received 231 specimens of lungs, preserved in alcohol, from small rodents trapped in Japan. Five of these were found to contain spherules typical of *Haplosporangium*. The rodent hosts and localities represented were as follows:

Eothenomys smithii smithii (Thomas). From Aomori, Kamikita Gun, Towada, 22 June 1955, 6 spherules in lung (Fig. 1).

Apodemus speciosus speciosus (Temminck). From Iwate Gun, Matsuo, 12 July 1955, 1 spherule in lung. From Miyagi, Ojika Gun, Kurumihana, 28 July 1955, many spherules in lung, at least 20. From

[1] From the U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Hamilton, Montana.



Haplosporangium parvum. FIG. 1. Optical section, whole mount, of spherule from lung of *Eothenomys*, Japan. 220 μ dia. Magnification 35 ×. FIG. 2. Microsection of portion of lung of *Ictonyx*, Africa. Magnification 80 ×. Photographs prepared by N. J. Kramis.

Ehima, Nii Gun, Sumino, 9 August 1955, 1 spherule. From Iwate, Iwate Gun, Matsuo, 12 July 1955, 1 spherule in lung.

Spherules found in lungs of these Japanese rodents varied from 150 μ to 350 μ in diameter and therefore were comparable in size to those found in mammals from Montana and Canada. Those found in lungs of Arizona rodents by Emmons and Ashburn (**1942**) were much smaller —i.e., 10 μ to 13 μ in diameter.

During the summer of 1956, a scientific working party organized by Harry Hoogstraal of the U. S. Naval Medical Research Unit No. 3, Cairo, Egypt, in cooperation with the East African Veterinary Research Organization collected many small mammals for museum purposes and for their ticks and other ectoparasites. Lung samples from 90 of these mammals were preserved in alcohol by Glen M. Kohls of the Rocky Mountain Laboratory, who was a member of the expedition. Typical *Haplosporangium* spherules were found in the lungs of two animals. One infected host was a rodent, *Cricetomys* sp., which came from the foothills of Mt. Meru, near Mulala, northeast of Arusha, Tanganyika, Africa. This lung specimen contained 3 large thick-walled spherules measuring 296 μ, 370 μ, and 370 μ in diameter, respectively. These large spherules were comparable in size to those found in rodents in Montana and Canada.

The other infected host, a small carnivore, *Ictonyx* sp., was collected about 20 miles northwest of Nairobi, Kenya, Africa. This lung contained hundreds of small spherules, ten of which measured 22 μ to 34 μ and averaged 27 μ in diameter (FIG. 2). These small spherules were more characteristic of the type found in rodents in southern United States.

Thus, haplomycosis in small mammals has now been reported from England, Japan, Europe, Asia, and Africa. As yet it has not been reported from Australia or South America. Haplomycosis has not been recognized as a human infection.

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—WILLIAM L. JELLISON, Rocky Mountain Laboratory.

A METHOD OF SEPARATING FUNGI FROM THE CULTURE MEDIUM

Following G. N. Bistis's note on a double-dish method of cultivating fungi (Mycologia **49**: 772–773. 1957), I offer an even simpler method for separating a fungus from the nutrient on which it is cultivated:

Prepare circular sheets of cellophane or filter paper equal in diameter to the inside of the bottom half of a petri dish. Autoclave them in a covered dish. Place one aseptically on the surface of solidified agar in each culture plate. Now inoculate the fungus on the cellophane or filter paper. It will grow normally on account of the diffusion of nutrient and agar through the cellophane or paper. Finally, take out paper and fungus. I think such a method will have all the advantages mentioned by Dr. Bistis.—S. R. BOSE, Department of Botany, Carmichael Medical College, Calcutta, India; at present at Laboratoire de Cryptogamie, 12, rue de Buffon, Paris Ve, France.

PHIALOPHORA RICHARDSIAE IN BRAZIL

In the Fall of 1953, a colony of *Phialophora Richardsiae* was isolated as an air-borne contaminant at the Instituto de Saude Publica, Salvador-Bahia, Brazil. To the authors' knowledge, this was the first time this species had been isolated in Brazil. This organism has been reported from Europe and North America as a cause of lumber and wood pulp blueing.[1, 2]

The original isolate from the United States was described as *Cadophora Richardsiae* Nannfeldt 1934.[1] Another isolate was described as *Cadophora brunnescens* Davidson 1935.[2] Conant[3] reduced the genus

[1] Melin, E. and J. A. Nannfeldt. 1934. Researches into the blueing of ground wood-pulp. Svenska Skogsvardfor. Tidskr. **32**: 397–616.

[2] Davidson, R. W. 1935. Fungi causing stain in logs and lumber in the Southern States, including five new species. Jour. Agric. Res. **50**: 789–807.

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Cadophora Lagerberg and Melin 1928 to synonymy with the genus *Phialophora* Thaxter 1915. The work of van Beyma [4] and Josefiak [5] confirmed the supposition that *Phialophora brunnescens* (Davidson) Conant 1937 was synonymous with *Phialophora Richardsiae* (Nannfeldt) Conant 1937.

Culturally, it was characterized by poorly developed aerial mycelium on Czapek-Dox agar and a luxurantly developed aerial growth on Sabouraud's dextrose agar. Microscopically, the aerial mycelium bore saucer-shaped phialide cups which formed large, spherical, thick-walled phialospores and narrow-funnel-shaped phialide cups which formed elongate phialospores.[5]—EUGENE J. JOSEFIAK, Department of Pathology of the Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, North Carolina; and MANUEL E. DA SILVA, Instituto de Saúde Pública, Salvador-Bahia, Brazil.

[4] van Beyma thoe Kingma, F. H. 1943. Beschreibung der im Centraalbureau voor Schimmelcultures vorhandenen Arten der Gattungen *Phialophora* Thaxter und *Margarinomyces* Laxa, nebst Schussel zu ihrer Bestimmung. Antonie van Leeuwenhoek **9**: 51–76.

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REVIEWS

YEASTS, by K. Arima, W. J. Nickerson, M. Pyke, H. Schanderl, A. S. Schultz, A. C. Thaysen, R. S. W. Thorne, and edited by W. Roman. 246 pages, 18 figs. W. Junk, the Hague, distributed in the western hemisphere by Academic Press, New York. 1958 (publication date given as Feb. 5, 1958; title page dated 1957). Price, $7.50.

This treatise deals with the various industrial applications of the species *Saccharomyces cerevisiae*, and with the single application of *Candida* (*Torulopsis*) *utilis*. Most of the contributors are more or less directly connected with the production of baker's yeast, food and fodder yeast, beer, wine, or sake. The biology and biochemistry of strains used in each industry are discussed, as well as plant procedures. Pertinent scientific contributions of the past few years are rather sparingly included.

This reviewer's first reaction to the book was that of disappointment. The editor's short introduction was long enough to reveal a smattering of knowledge about yeasts and to indicate a low standard of writing. Typographical errors and misspellings are numerous and the type selected is too small for easy reading. Although it is merely amusing to learn that Barker is credited with reporting cytological work on yeasts in the year 1091 (p. 43), such errors involving metric units (p. 20, table 7) are more serious. According to statements on the jacket, this book is the first in a series presenting both experimental and processing aspects of various industries based on biology. Unless editorial and publishing standards are raised in subsequent volumes, it seems to the reviewer that difficulty will be experienced in persuading outstanding people to contribute.

Fortunately, most chapters are informative, and one who reads the book is wiser for the experience. Nickerson and Schultz present interesting information on general cytology and physiology of baker's yeast. Nickerson makes an issue of his doubt that polyploidy exists in yeasts, partly as a result of the very poor cytological work reported by Subramaniam and associates on the number of chromosomes in supposedly polyploid forms of *S. cerevisiae*, and partly because of Abrams's report that acenaphthene-treated yeast had no more deoxyribonucleic acid than untreated cells. The error here, most likely, was the assumption that the particular cell types obtained by acenaphthene-treatment in this

study were polyploid simply because they were larger than the untreated strain. Nickerson was evidently unaware of the excellent agreement reported by Ogur and associates in 1952 for relative amounts of DNA in a polyploid series of *Saccharomyces*. The ratios were 2.26, 4.57, 6.18, and 9.42, respectively, for haploid, diploid, triploid, and tetraploid cells.

The reviewer's knowledge of wine fermentation is not so great that he can adequately judge Schanderl's contribution, but his unqualified statements regarding taxonomy and genetics of yeasts are most intriguing. We learn that the genus *Schizosaccharomyces* really consists of bacteria, and that it contains only one species, *S. pombe*, which we are told exists as haploid, diploid, and tetraploid forms, and that the haploid differs from the diploid and polyploid forms not only morphologically but biochemically. The haploid form ferments saccharose easily whereas the polyploid form does not. Since the sexuality of *Schizosaccharomyces* is typical of yeasts, we must assume the bacteria, not the yeasts, are the simplest forms of the Ascomycetes. Schanderl reports that in *Brettanomyces* small and large cells copulate to produce polyploids. It is regrettable that he gives no references to this new and refreshing knowledge. Biochemists will also be amused to read that wine yeasts "do not require added growth factors, which are produced by the yeasts themselves, nor addition of nitrogenous substances, since wine yeasts are able to assimilate molecular nitrogen directly from the air." There is a grain of truth here, greatly magnified.

On the credit side for the book are the interesting and informative chapters by Pyke, Thorne, and Thaysen, who conscientiously covered analysis of the cell, its biochemical reactions, and industrial use. Also worthy of mention is the table of contents, given in detail in outline form. This arrangement allows one to easily find any topic he may wish to read again.—LYNFERD J. WICKERHAM.

THE MUSHROOM HUNTER'S FIELD GUIDE, by Alexander H. Smith. ii + 197 pp., 155 figs. University of Michigan Press, Ann Arbor, 1958. $4.95.

Early in this year, I received from California a package of dried, thin-sliced boleti with a request that I identify the species that had caused serious illness to several people. At about the same time, I had an inquiry, likewise from California, concerning books that would be of assistance to a person who wished to start out collecting mushrooms for his own eating or for sale. These two requests are only the latest examples of what appears to be an increasing interest on the part of the

public in learning something of the larger fungi, if not of their use for the table. The books available to the layman have never given complete satisfaction—they were either too complicated and technical or too brief and diffuse. At last, however, it appears that we have something that we can recommend with the confidence that it will perform a service.

Smith's *Field Guide* is only what it says it is. It is not a compendium or a conspectus of the mushrooms. Its purpose is not primarily to enable one to make scientific identifications, but mainly to provide protection against serious mistakes. The excellently written introductory chapter gives general information concerning the larger fungi and their characteristics and structure, the precautions to be exercised, and complete advice on collecting and using them.

As likewise stated in the introduction, the book includes forms the beginner is most likely to find, the truly fine edible mushrooms whether common or not, and the dangerous ones. The coverage is mainly the northern expanse of states in this country from coast to coast, not so complete for the southern states. The inclusion of the Pacific Coast states is a welcome feature, in the light of recent complaints made to this reviewer. Of the 124 species included, somewhat over half are agarics, and the remainder morels, puffballs, boletes, hydnums, corals and polypores. All the species are amply illustrated, mostly natural size or nearly so, among the 155 roto-print reproductions of superb photographs—as the author says, the backbone of the volume.

Additional assistance is provided by special lists of species—for the western states, those safe for beginners, those associated with different trees, and species that appear at the different seasons of the year. Keys are presented for the species of the various groups. A useful glossary is appended.

The arrangement of the volume is convenient. For the most part, there is one species to the page, with discussions of when and where to find it, the important field characteristics in non-technical language, and its edibility or harmfulness, with the excellent, good-sized illustration at top or bottom of the page. In order to accommodate this presentation, the book is about a foot tall, about half a foot wide and three quarters of an inch thick—not too large for a pocket of reasonable size, although of course a little bulky. It is a pleasing, attractive book, with a beautiful reproduction of *Coprinus comatus* on both covers, well printed, with a good "feel," and apparently very ruggedly put up.

This reviewer is delighted to have this book available. Perhaps now he will not receive the customary response that his recommended publications are "over my head" or "don't tell me anything."—WALTER H. SNELL.

MANUSCRIPT

Publication in MYCOLOGIA is ordinarily restricted to those who have been members in good standing of the Mycological Society of America for over a year immediately preceding submission of manuscript. Exceptions to this regulation require a favorable vote by a majority of the Editorial Board. When a paper has two or more authors, the person submitting the paper is expected to be a member.

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